EVOLUTION OF REPRODUCTIVE TRAITS UNDER PRE-AND POST-MATING SEXUAL SELECTION

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ABSTRACT

SUMIT DHOLE: EVOLUTION OF REPRODUCTIVE TRAITS UNDER PRE-AND POST-MATING SEXUAL SELECTION
(Under the direction of Maria Servedio)

Sexual selection has shaped the evolution of a variety of reproductive traits in males and females of numerous species. Because females of most animal species mate with multiple males, sexual selection can extend beyond mate choice and inter-sexual competition for mates to post-mating events such as sperm competition and cryptic female mate choice. In this thesis I addresses the evolution of reproductive traits under selection before and after mating. In Chapter 2, I address the evolution of female choosiness and male mating displays that function as indicators of male genetic quality. I address the influence on the evolution of these reproductive traits of female ability to evaluate male genetic quality without recourse to male displays. Counter to intuition, I find that direct detection of male quality by females, instead of impeding, can facilitate the evolution of male displays at intermediate levels of detectability. I present a new continuum framework for different mechanisms of indicator displays that heretofore have been modeled as discrete types. I find that the continuum framework reveals interesting patterns in how direct detectability of male quality influences the evolution of different types of indicators.

In Chapter 3 I investigate age-dependent plasticity in male mating investment using *Drosophila pseudoobscura*. I find that male mating investment generally increases with male age, and intermediate-aged males are most discriminatory with respect to female age, making smaller investments when mating with older females. Male mating investment was correlated

with fitness payoffs from matings, but matings with young females were more profitable for males than matings with old females.

In Chapter 4 addresses the evolution of male seminal fluid composition. I investigate how males evolve to allocate resources towards different seminal fluid proteins that increase male sperm-competitive fitness in different ways. I find that the relative efficiencies of proteins play a large role in determining the evolutionarily stable ejaculate composition. Also, plasticity in ejaculate composition can contribute to the maintenance of genetic variation in ejaculate composition across populations.

Together these chapters form important stepping stones for designing models that address the interactions and coevolution of reproductive traits that function before and after mating.

To my mother,
from who I inherited the curiosity about the natural world,
and without whose encouragement I would not have been a biologist.

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CHAPTER I: GENERAL INTRODUCTION

"Of the branches of biological sciences to which Charles Darwin's life-work has given us the key, few, if any, are as attractive as the subject of sexual selection." (Fisher 1915). Besides the fact that sexual selection is associated with some of the most baroque traits seen in organisms, another reason why so many evolutionary biologists seem to share Fisher's view is that sexual selection remains the most robust evolutionary explanation for a tremendous diversity of reproductive traits. For over a century after its inception (Darwin 1871), sexual selection remained a small sub-field of evolutionary biology and was exclusively associated with mate choice and competition for acquiring mates. Over the past four decades it has become apparent that in a large number of species sexual selection is not limited to pre-mating events. But it can extend beyond mating up to fertilization through phenomena such as sperm competition and post-copulatory mate choice (sperm selection) (Birkhead and Møller 1998; Simmons 2001). Thus, reproductive success of individuals is determined by traits that function both before and after mating. The chapters in this thesis address sexual selection acting on reproductive traits that function at different stages of reproduction. Chapter 2 addresses the evolution of female mate choice and of male displays, which influence the mating success of males. Chapters 3 and 4 address post-mating selection on male reproductive traits that influence the fitness outcome of the matings that males may acquire.

While chapter 3 includes empirical work, chapters 2 and 4 present mathematical models that are based on abstract simplifications of biological systems. The purpose of such

models (sometimes termed “proof-of-concept models”; Servedio et al. [in press]) is not always to produce quantitative predictions that can be immediately tested in an empirical system. The goal of these models is usually to test the logic of verbal evolutionary explanations, or to provide baseline expectations for how traits would evolve in absence of specific biological complexities, and thus to serve as a “yard stick” for more complex empirical or theoretical studies (Servedio et al. [in press]). Moreover, even such simple models often reveal phenomena and patterns that may be missed by verbal evolutionary arguments. Neither of the models presented in these chapters was designed to yield predictions that can be tested in a specific system. Yet, both models reveal interesting, and some unintuitive, biological patterns that may be sought empirically across multiple species. Furthermore, both models can serve as stepping-stones for designing models that are tailored to address questions on sexual selection in specific empirical systems.

Elaborate male mating displays, ranging from behavioral “performances” to extravagant morphological structures, are common in a wide variety of taxa (Andersson 1994; Birkhead and Møller 1998). Many of these traits reduce viability of males that produce them, and thus their evolution can’t be explained by natural selection. Darwin (1871) was the first to offer an evolutionary explanation for such traits. He proposed that male displays evolve because females preferentially mate with males that exhibit elaborate displays. Although elaborate male displays can be explained with such straight-forward logic, Darwin could not offer an evolutionary explanation for female mate choice itself. Indeed, exhibiting mate choice is likely to incur costs on females: choosy females may need to spend more time and energy searching for their preferred mates, may expose themselves to predation and other hazards while searching for preferred mates. Yet, females are often very choosy with regards

to the males they mate with (Jennions and Petrie 1997; Kokko et al. 2003). Thus, often the more difficult and interesting question is why females evolve mate choice favoring male displays.

In certain species, the costs of mate choice are offset by direct benefits that females gain by mating with ornamented males, such as nuptial gifts, protection, territory, resources etc. In many species, however, males only provide genetic material for the offspring. How can costly female mate choice spread in such cases? Fisher (1930) offered the first explanation: an inevitable consequence of female mate choice is that the sons of choosy females are more likely to be attractive, if male attractiveness is heritable. Alleles for mate choice can then spread in the population indirectly through the more attractive sons. However, the "Fisherian mechanism" does not always lead to the evolution of strong female preferences and large male ornaments when mate choice is costly. Another, now widely used, explanation is that elaborate displays indicate high heritable quality of a male (often termed "good genes"); by preferentially mating with males that have elaborate displays, females can acquire the high quality genes for their offspring (Zahavi 1975; Maynard Smith 1985, 1991; Kokko et al. 2006). A large amount of theoretical and empirical work has addressed the evolution of displays that may function as indicators of good genes (reviewed in Andersson 1994; Kokko et al. 2003, 2006; Mead and Arnold 2004; Andersson and Simmons 2006).

In Chapter 2, we make three novel contributions to the study of indicator displays. First, we address addresses the effect of direct detectability of male quality on the evolution of indicator displays and of female choosiness. One important assumption common to previous studies of indicators of good genes has been that male genetic quality itself is not directly detectable by females. Direct detection of male quality has been assumed to impede

evolution of indicator displays and of female mate choice favoring such displays. We show that direct detectability can actually facilitate the evolution of male displays under a broad range of biological conditions.

Second, unlike most models of indicators of good genes, we address evolution of female *choosiness* instead of female *mating preference*. The difference between these is subtle, but critical: a mating preference is a bias towards a particular male trait, while choosiness is the degree of bias that females express. Considering the evolution of female choosiness reveals a number of patterns that otherwise could not become apparent.

Third, in this model we present a new framework that accommodates the different mechanisms that can result in honest indication of the quality by male displays on a continuum. The continuum framework accommodates a wide range of biologically possible set of indicator mechanisms and also allows direct comparison between different indicator mechanisms.

While such pre-mating traits determine the likelihood of mating for different males, the fitness outcome of the matings is often strongly dependent upon male traits that function after mating. One important male trait that plays a role after the mating is the male's ejaculate, which often has a complex composition with numerous substances in addition to sperm. How males allocate their resources into individual ejaculates, and the composition of those ejaculates, can have a large influence on male fitness. When resources are limiting, males may benefit by adjusting their investment in ejaculates depending upon the potential fitness payoff expected from a mating. Facultative adjustment in resource allocation however would be beneficial only when future opportunities for reproduction are likely. Age is an

important factor that determines the likelihood of future reproductive opportunities. Chapter 3 discusses an experiment used to address the effects of male and female age on male mating investment, and its potential fitness consequences for males in *Drosophila pseudoobscura* (Dhole and Pfennig 2014a). We find that the total male mating investment in individual matings as well as the degree of facultative adjustment by male in their mating investment is influenced by male age and female age. We also find that male and female ages interact to form a complex pattern of age-dependent mating investment that can affect age-specific fitness for both males and females.

Male ejaculates are complex, with different components of the ejaculate performing different functions. Therefore, in addition to the total investment in an ejaculate, the relative investments in different components of the ejaculate can influence male fitness. Seminal fluid proteins (Sfps) form a large fraction of male investment in ejaculates (Poiani 2006; Simmons 2001). A number of Sfps are known to play an important function in sperm competition. Moreover, different Sfps influence male success in sperm competition in different contexts. For example, certain Sfps transferred by males delay female's remating for a short period after the mating, aiding the male in avoiding sperm competition with the subsequent male that the female would mate with. Other proteins are known to aid males in removal of pre-existing sperm from female's reproductive tract, while some proteins aid males in defending their sperm from subsequent males. Thus, Sfps that increase the fitness of a male mating with a previously mated female may not be beneficial to a male mating with a virgin female. In Chapter 4, we present a mathematical model that asks how a male should distribute a limited set of resources across three functional categories of proteins, given that males may mate with either virgin or previously mated females. We also address the

influence of plasticity in ejaculate composition. We find that different compositions can be maintained across populations when males can adjust their investment in different Sfps based on the mating status of the female that they encounter. This chapter presents the first formal theoretical treatment of the evolution of seminal fluid composition, a trait that can have a large influence of male fitness.

Together these chapters form stepping-stones towards an integrative approach to studying pre-and post-mating male traits and their coevolution.

CHAPTER II: DIRECT DETECTION OF MALE QUALITY CAN FACILITATE THE EVOLUTION OF FEMALE CHOOSINESS AND INDICATORS OF GOOD GENES: EVOLUTION ACROSS A CONTINUUM OF INDICATOR MECHANISMS.

Summary

The evolution of male mating displays as indicators of male quality and female mate choice favoring such indicators has been the subject of extensive theoretical and empirical research for over four decades. Yet, much debate exists about whether these reproductive traits of males and females can evolve through such indirect benefits of female mate choice. Here we use a population genetic model to address how the extent to which females can directly detect male quality influences the evolution of female choosiness and of male displays. We also present a continuum framework for the different indicator mechanisms that are traditionally modeled separately: condition-dependent, revealing and pure epistatic. In addition to allowing access to potentially more realistic combinations of indicator mechanisms, this framework allows a more direct comparison between different indicator mechanisms than was previously possible. Counter to intuition, we find that direct detection of male quality can facilitate, instead of impede, the evolution of female choosiness and male displays, at low and intermediate levels of detectability. We also find that direct detection of male quality can greatly alter the relative ease with which male displays can evolve under different indicator mechanisms. Our results have important implications for empirical research seeking to identify indicators of good genes.

Introduction

One widely employed explanation for the evolution of female mate choice and male mating displays is that females gain high quality genes for their offspring by mating with males with exaggerated displays. If male displays are correlated with alleles that impart higher fitness, choosy females are more likely to produce offspring with higher fitness (Fisher 1915; Andersson 1986, 1994; Pomiankowski 1988). Much theoretical and empirical research has focused on male displays correlated with male genetic quality, asking questions including how these displays evolve (Kokko et al. 2003; Mead and Arnold 2004; Andersson and Simmons 2006; McLean et al. 2012; Prokop et al. 2012) and how an honest correlation between male display and quality is maintained in the face of incentives for males to deceive females about their quality (Grafen, 1990; Zahavi & Zahavi, 1997; Emlen et al, 2012).

The main premise of this explanation for the evolution of male displays and female mate choice favoring them is that females cannot directly detect male quality, and thus they must rely on the male displays as indicators of "good genes". However, empirical evidence suggests that females can possess the ability to directly detect male quality (e.g. Thom et al. 2008). For instance, the Hamilton and Zuk model (1982) of good genes proposes that male displays may indicate genes for immune competence. Indeed, many diseases have perceivable symptoms, which may allow females to directly detect males that are susceptible or resistant to a particular disease. Body size is another example of an important component of condition that is easily perceptible.

Direct detection of male quality by females has been supposed to disfavor the evolution of indicator traits: if females can accurately assess the quality of potential mates without recourse to displays, it seems logical that selection will act against males that produce costly displays. One problem with this line of reasoning is that it does not take into

account the possibility of imperfect direct detection of male quality: if females have only a moderate ability to directly detect male quality, and could improve their accuracy in choosing high-quality mates by using additional information, then selection could favor the evolution of a display indicating male quality. For example, male displays may aid in female evaluation of male body size. But even with the inclusion of imperfect detection, the above logic leads to the intuition that evolution of indicators of good genes is most favored by complete absence of direct detection of male quality, with imperfect direct detection only being less constraining than perfect direct detection.

In this paper we address how female ability to directly detect male condition without recourse to male display influences the evolution of male indicator displays and of female choosiness. Note that we consider the evolution of choosiness (discussed more below), instead of a specific preference, as choosiness can influence preferences over multiple male traits. Our model makes two novel contributions. First, we show that imperfect direct detection of male quality by females can actually favor the evolution of indicator displays and of costly female mate choice. Second, we present a continuum structure for the three classic types of indicator displays: condition dependent, revealing and pure epistatic (Andersson 1994). These indicator mechanisms are commonly modeled separately, but in reality are not mutually exclusive. The continuum allows us to analyze how direct detectability of condition affects indicator displays that function through the three different mechanisms as well as through all possible combinations thereof.

Female mating preference versus choosiness

Most theoretical studies of mate choice evolution model the evolution of a new female *mating preference* (a bias towards a specific male trait) from random mating (e.g. Kirkpatrick 1982; Pomiankowski and Iwasa 1993; Servedio and Lande 2006; reviewed in Kuijper et al. 2012; but see Bleu et al. 2012; Etienne et al. 2014) Often, though, the female trait that is evolving may be the *strength of* a pre-existing female preference, that is, female choosiness. Indeed, the evolution of higher choosiness may sometimes appear indistinguishable from the evolution of a new preference, especially when a very weak preference already exists and females evolve to be more choosy. Yet, the distinction between preference and choosiness is important, because the loci that control the preference (direction of bias) are likely to be different from the loci that control the strength of that preference (choosiness). Moreover, choosiness is likely to be, at least partially, controlled by loci that influence the nervous system at a broad level, and affect female responses towards a multitude of stimuli, including towards different male traits (Appeltants et al. 2002; Vyas et al. 2008; Pawlisch and Riters 2010). In this paper we model the evolution of female choosiness at this broad level, where higher choosiness increases the strength of multiple female preferences. Specifically, we model the evolution of a choosiness allele that influences the strength of female preference toward male displays as well as toward male condition. We recognize that female choosiness regarding different male traits may be independent to different levels in different real biological systems. However, the relevance of our model for empirical systems does not require that *all* loci that influence female choosiness in a species affect female behavior towards all male traits simultaneously. Our model only requires that at least one of the loci affecting female choosiness influences female behavior towards multiple male traits.

A continuum of indicator mechanisms

Three mechanisms are generally recognized for indicators of good genes – condition-dependent indicators, pure epistatic indicators (also called Zahavi's handicap mechanism) and revealing indicators. The mechanisms differ from each other in the expression of the display by males in low condition (and in turn, female preference for those males), and in the marginal costs of producing the display incurred by males in low condition. Most models of good genes indicators address the three mechanisms separately (Andersson 1986; Kirkpatrick 1996; Van Doorn and Weissing 2006; Kuijper et al. 2012; Tazzyman et al. 2014). However, these mechanisms need not be mutually exclusive, and multiple mechanisms may act simultaneously in many real biological systems. Our model is parameterized so that the classically recognized indicator mechanisms are points on a continuum. This not only allows us to examine all possible combinations of the three indicator mechanisms, but also allows us to compare the three types of indicator mechanisms more directly to each other.

Here we remind the reader of the three classic mechanisms, and in the next section describe how a continuum exists between them with the three mechanisms falling at the edges of the continuum in parametric space. We follow the definitions of these mechanisms as summarized by Andersson (1994):

1. *Condition-dependent indicators* are displays that are produced by males only when they are in high condition. Low condition males never produce a display and thus never pay a viability cost for displaying. In addition to such extreme case of 'complete condition-dependence', a quantitatively varying display is termed 'partially condition dependent' if males in low condition produce a smaller display, pay

proportionally smaller costs for it (i.e. identical marginal cost) and have a proportionally smaller mating advantage.

2. *Revealing indicators* are displays that are produced by males of all conditions. Thus, all males carrying the allele for the ornamental display pay the full cost of producing the display. However, the displays produced by low condition males are of a visibly lower quality and are not preferred by females. Low condition males thus do not gain any mating advantage by producing the display.

3. *Pure epistatic indicators* are also displays that are produced by males of all conditions, but they are of similar quality in all males. As the displays are identical between high and low condition males, they confer equal mating advantage. However, low condition males pay a higher marginal cost for the display compared to high condition males. The correlation between male display and male quality in this mechanism arises due to disproportionately lower survival of low condition males when they produce a display

We use a population genetic model to study the evolution of both female choosiness (on a background of random mating) and a male display. We incorporate a continuum framework for indicator mechanisms to directly compare evolution across all possible combinations of indicator mechanisms. We address two questions with our model – 1) How does direct detectability of male condition influence the evolution of male displays and of female choosiness?, and 2) does direct detectability of condition influence which indicator mechanisms more easily allow male display and choosiness to spread in the population?

We show that when female choosiness is allowed to evolve, low to intermediate levels of direct detectability of condition actually facilitates the evolution of indicator

displays instead of impeding it. At high levels, however, direct detectability of condition does impede the evolution of indicator displays. We also show that imperfect direct detection of male quality can alter which type of indicator displays are more feasible to evolve. As certain male genetic qualities are more likely to be directly detectable by females (e.g. disease resistance, body size), our results have important implications for empirical research seeking to identify examples of indicators of good genes.

Model

A three-locus structure has been widely employed by other researchers to study the evolution of indicators of good genes over the past three decades, with one locus each controlling the expression of male display, female mate choice and "good genes" or condition (Maynard Smith 1976; Andersson 1986, 1994; Kirkpatrick 1986; Tomlinson 1988). We use a modification of this basic three-locus model to incorporate direct detectability of condition, as well as to incorporate the three indicator mechanisms in a single continuum framework. A key novel feature of our approach is a parameter (γ) expressing the extent to which females can directly detect male condition. Our model also includes two parameters (β and σ, see below) that allow the indicator mechanisms to be studied as part of a continuum.

Individuals in our model have three loci: the H locus controls the level of choosiness in females; the T locus controls the ability of males to produce a display; and the C locus controls genetic quality, also referred to as 'condition', in both males and females. Each of the loci can contain one of two alleles, designated by the subscripts 1 and 2. This gives eight genotypes $H_1T_1C_1$, $H_1T_1C_2$, $H_1T_2C_1$ and so on, referred to as X_1 through X_8, with their respective genotypic frequencies denoted x_1 through x_8.

Viability selection

The condition locus (C) is expressed in both sexes, giving C_2 individuals a relative viability advantage (s_c) over C_1 individuals. The display locus (T) is expressed only in males. Only males with the T_2 allele are capable of producing the display. When males produce a display, they pay a viability cost (s_t). For indicator mechanisms in which males in low condition can express the display (revealing, epistatic or partially condition-dependent indicators), the parameter σ determines the cost of the display incurred by males in low condition relative to the cost incurred by males in high condition ($0 \leq \sigma \leq 1/s_t$). The parameter σ is thus a modifier of the cost of the display for low condition males. The relative viabilities of males for the three types of indicator mechanisms are summarized in Table 1. The exact equations can be found in the Appendix 2.1.

The H locus is expressed only in females. Females with the H_2 allele are choosy with regards to their mating preferences, whereas females with the H_1 allele are not choosy and mate randomly. Females with the H_2 allele pay a search cost for being choosy, which depends on the frequency of their preferred males. Females pay the maximum search cost (given by the parameter s_h) when their preferred males are absent, and this cost decreases as the preferred males become more common in the population. Females with the H_2 allele thus have a viability of $1 - s_h^*$, where s_h^* is the search cost weighted by the frequency of the preferred males and the level of choosiness (see below for exact definition of s_h^*). Viability selection on females thus depends upon the level of choosiness and the ease with which they can access their preferred mates.

Sexual selection

We model evolution in a polygynous population, where males of different genotypes can differ in their mating success, but all females that survive after viability selection (due to search costs) are able to mate and have equal fecundity. H_2 females are $1+a$ times more likely to mate with a male that has both the high condition allele (C_2) and the display allele (T_2) relative to males that lack both these alleles (T_1C_1 males), if females encounter one male of each type.

Unlike previous models of indicator mechanisms, ours includes direct detectability of male condition by females, which can vary continuously between complete absence of direct detection and perfect direct detection of male condition. This allows us to analyze cases where females can gather perfect information about a male's condition without recourse to the male display, as well as cases where the male display aids females in assessing the condition of males, which may be only partially detectable in absence of the display. We compare the evolution of the male display and female choosiness under the spectrum of all possible levels of direct detectability of male condition to the traditionally considered case where male condition is invisible to females. The parameter γ controls the visibility of male condition to the females when males do not produce a display (T_1 males). Females are $1+\gamma a$ times more likely to mate with T_1C_2 males than T_1C_1 males, if they encounter one of each. When condition is not directly detectable by females ($\gamma = 0$), females cannot discriminate between T_1C_2 and T_1C_1 males. Traditional models of good genes indicators so far have only considered this case without direct detectability of condition. When condition is completely visible ($\gamma = 1$), females pay attention only to male condition, and do not discriminate between high condition males based on the display (i.e. females are $1+a$ times more likely to mate

with both T_1C_2 and T_2C_2 males). Another way to phrase the biological meaning of the parameter γ is that it determines how much information the male display adds to female evaluation of male condition. On one end of the continuum ($\gamma = 0$), the male display is the only indicator of male condition, which otherwise is undetectable. Intermediate values of the parameter ($0 < \gamma < 1$) mean that females can detect male condition without the display, but the display enhances female evaluation of male condition. On the other end of the continuum ($\gamma = 1$), the male display does not add any information to females' evaluation of male condition.

The parameter β controls the likelihood of choosy females mating with ornamented males in low condition (T_2C_1) relative to unornamented males in low condition (T_1C_1). Females are $1+\beta a$ times more likely to mate upon encounter with T_2C_1 males compared to T_1C_1 males. β is thus a modifier of the mating advantage gained by low condition males with a display. In our model, β and σ (modifier of display cost) help to define the three main types of indicator mechanisms as follows (Table 1). For completely condition-dependent indicators, $\beta = 0$ and $\sigma = 0$, because males in low condition do not produce a display. For revealing indicators, $\beta = 0$ and $\sigma = 1$, because males in low condition produce a costly display that is visibly lower in quality than displays produced by males in high condition. For pure epistatic indicators, $\beta = 1$ and $1 < \sigma < 1/s_t$, because low condition males produce displays identical to those produced by high condition males, but they pay higher marginal cost for doing so. Thus, depending upon the biology of a specific system in nature, values of β smaller than 1 may indicate either lower quality or smaller size (or both) of the display produced by low condition males compared to high condition males. A lower β due to lower quality of the display need not reduce the cost of making the display (σ need not be smaller

than 1) for low condition males (such as in the case of revealing displays). However, in systems where the value of β is reduced due to males in low condition making smaller displays, these displays are likely to be less costly ($\sigma < 1$; such as in the case of complete condition dependence; see the *Continuum of indicator mechanisms* section below).

Cost of choosiness for females

As mentioned above, females with the H_2 allele pay a search cost for being choosy, which depends on the frequency of their preferred males. This can now be defined. The cost decreases as the preferred males become more common in the population. Following Otto et al. (2008), the weighted cost of mate choice (search cost) is given by

$$s_h^* = s_h - \frac{s_h z}{(1+a)} \tag{1}$$

where

$$z = \left(x'_{T_1C_1} + (1+\gamma a)x'_{T_1C_2} + (1+\beta a)x'_{T_2C_1} + (1+a)x'_{T_2C_2} \right).$$

Here $x'_{T_iC_l}$ is the frequency after natural selection of males with alleles T_i and C_l at the corresponding loci.

Following viability selection and non-random mating according to the scheme described above, free recombination precedes zygote formation. We generate recursion equations for the genotype frequencies and transform these into equations for the allelic frequencies at the three loci, and for the first and second order linkage disequilibria. As detailed below, we use numerical iterations of these recursion equations to ask under what condition the alleles for the display and for female choosiness can spread in the population from low initial frequencies.

Continuum of indicator mechanisms

Although the three classic indicator mechanisms are generally modeled separately, they are not mutually exclusive. For example, low condition males may have to pay a higher marginal cost for producing a display ($\sigma > \beta$, epistatic mechanism) and yet produce a low quality display that females can recognize and not prefer ($\beta = 0$, revealing mechanism). Our modeling framework allows us to examine all of these mechanisms as part of a continuum formed by combinations of different values of the parameters σ and β (Figure 1). This continuum includes all possible combinations of the three classic mechanisms as well as male displays that are completely independent of male condition (and thus not indicators of male quality; β=1, σ =1). A benefit of this continuum structure is that it recognizes that the three classical mechanisms are simply points in a much broader framework, as well as that it allows us to directly compare how easily different indicator mechanisms allow evolution of male displays and of female choosiness. This framework also allows us to directly address how direct detectability of condition influences evolution through different indicator mechanisms.

Analysis

Our model is designed to address two questions – 1) how does direct detectability of male condition influence the evolution of male displays and of female choosiness?, and 2) does direct detectability change which indicator mechanisms more easily allow male display and choosiness to spread in the population?

We address the first question by focusing only on the three classic indicator mechanisms – complete condition-dependent, revealing and pure epistatic (Figure 1). We then use the continuum framework to address the second question. In addition to numerical simulations, we also use the multilocus notation of Barton and Turelli (1991) to extract selection coefficients that allow us to determine the strength and sources of selection acting on the male display, on female choosiness and on condition itself (see Appendix 2.2 for details). This enables us to separate out and compare the direct versus indirect selection acting on the three loci (Barton and Servedio, in revision).

In the absence of a mechanism to create variation at the condition locus, the C_2 allele will quickly reach fixation in the population. In natural populations, condition is likely to be determined by a large number of loci, mutations at which are thought to maintain variation in male condition (Houle 1992; Tomkins et al. 2004). The evolution of condition is not a focus of our model, yet variation at the C locus is critical for females to benefit by acquiring "good genes" for their offspring through mate choice. Therefore, for the numerical simulations, we hold the frequency of the C_2 allele constant at 0.5, but allow the evolution of the rest of the loci and linkage disequilibria to proceed (they take on approximate values to those from exact iteration; see the Appendix 2.1 for details). This approach has an effect analogous to the mutation bias method used in previous quantitative genetic models of good genes indicators (e.g. Iwasa et al. 1991; Houle and Kondrashov 2002; Tazzyman et al. 2014). *Mathematica* code for all the analyses and for generating the figures can be found in the online supplementary material.

Results

Evolution of female choosiness

When condition is not directly detectable ($\gamma = 0$), females across all three classic indicator mechanisms evolve to be choosy only when the cost of choice is low (Figure 2). With increasing detectability of condition (increasing γ), however, female choosiness can spread in the population under all three indicator mechanisms even when the cost of mate choice is high. Part of the reason for this effect is that as the detectability of male condition increases, choosy females can recognize and access high quality males without the display (T_1C_2 males in addition to T_2C_2). As the search cost of mate choice decreases with access to preferred mates, higher γ reduces the weighted search costs of mate choice (See Appendix 2.3).

In mating systems where females do not receive direct benefits through mate choice, higher choosiness can evolve only through indirect selection, that is, selection that results from the association (linkage disequilibria) of alleles for higher choosiness with alleles for higher male mating success and/or with alleles of higher genetic quality (Lande 1981; Kirkpatrick 1982; Kokko et al. 2003). With increasing γ, alleles for female choosiness evolve a stronger association with alleles for higher genetic quality. Thus, increasing direct detectability of male quality results in more evolution of the choosiness allele through indirect selection (Figure 3B), resulting specifically from the selective forces acting on condition (Figure 3C). In contrast, evolution of female choosiness due to indirect selection through the display trait is quite low, and decreases as direct detection of condition increases (Figure 3D). Thus, with increasing detectability of condition, indirect selection through the display may play only a small role in evolution of female choosiness. Part of the reason for this is that initially when the male display is rare in the population, the low genetic variation at the T locus limits evolution of a large linkage disequilibrium between choosiness and

display. A much larger linkage disequilibrium may build up between choosiness and condition (held fixed at frequency of 0.5). We believe comparing the effect of indirect selection on choosiness through display and condition at different frequencies is a biologically more appropriate – displays are unlikely to reach high frequencies in absence of female choosiness. This pattern remains qualitatively similar and becomes even more striking when the effects of indirect selection through the display and condition are compared with initial frequency of the display set at 0.5 (results not shown) – indirect selection through condition plays an even larger role compared to that through the display.

Evolution of male displays

As the viability cost of male display increases, male displays become less likely to evolve across all indicator mechanisms (Figure 4). The nature of the effect (facilitating versus inhibiting) that increasing direct detectability of condition has on the evolution of male displays depends on its exact value. When male condition is completely visible to females ($\gamma = 1$), male displays do not evolve under condition-dependent or revealing mechanisms, irrespective of their cost (Figure 4 A & B). However, with complete direct detectability of condition, pure epistatic indicators may still evolve. This is because unlike in the case of the other two types of indicator mechanisms, with the pure epistatic mechanism, displays can give a mating advantage when males are in low condition. At very high levels, direct detectability of condition impedes the evolution of male displays, allowing displays to evolve only when their viability costs are low (Figure 4). At low to intermediate levels, however, direct detectability of male condition facilitates the evolution of costly male displays for all types of indicator mechanisms (Figure 4). That is, with low to intermediate

detectability of condition, male displays can evolve in spite of costs that impede evolution of male displays when condition is completely undetectable.

At all levels, as the detectability of condition increases (increasing value of γ), discrimination by females between T_1C_2 and T_2C_2 males decreases, thus lowering the mating advantage that displaying males gain with choosy females. *For a given frequency of choosy females*, the net strength of direct natural and sexual selection favoring the male display thus becomes smaller with increasing direct detectability of condition (Figure 5). The effect of direct natural and direct sexual selection cannot be further separated from each other. However, γ only affects the difference in mating advantage gained by having high condition and the display together versus having only high condition; γ does not affect the cost of a display. Thus, the reduction in direct selection favoring the display is due only to the reduction in net sexual selection on the display.

The net sexual selection acting on the male display is a function of both the mating advantage gained through a display as well as the frequency of choosy females who confer that mating advantage. As described above, direct detectability of condition allows female choosiness to reach a higher frequency in the population. The effect of the reduction in mating advantage of the display due to increasing γ can be thus be counteracted by the spread of female choosiness with increasing γ. Direct detectability of condition thus reduces the mating advantage gained by males through the display, but increases the number of females in the population that give them the mating advantage, the latter of which accounts for the large areas of display evolution in Figure 4.

Continuum of indicator mechanisms

Below we first describe how the different indicator mechanisms influence the evolution of male indicator displays in absence of direct detectability of condition. Then we discuss how adding direct detectability of condition influences the effect of the different indicator mechanisms on the evolution of male display. One goal here is to compare the *relative* ease with which a male display can spread under the different indicator mechanisms that form the continuum. A second goal is to determine whether the pattern of relative ease changes with detectability of condition.

When male condition is not directly detectable, the male display fails to spread under any mechanism across the continuum (Figure 6D), unless female choosiness is strong enough (Figure 6A). Without direct detectability of condition, male displays are easiest to evolve when the only indicator mechanism involved is complete condition-dependence (see Figure 1), that is, when low condition males do not produce a display at all (Figure 6A). The evolution of male displays becomes more difficult with decreasing levels of condition-dependence ($0 < \sigma = \beta < 1$; Figure 6A), that is, with decreasing difference between the size of displays produced by males in different conditions. In agreement with previous models (Pomiankowski 1988; Kuijper et al. 2012; Tazzyman et al. 2014), we find that in absence of direct detectability of male condition, pure epistatic indicators ($1 < \sigma < 1/s_t$; $\beta = 1$) are unlikely to evolve, or are more difficult to evolve than condition-dependent and revealing indicators (Figure 6A).

At low levels of direct detectability of condition ($\gamma = 0.2$), male displays can spread in the population under a wider range of indicator mechanisms compared to the case of no direct detectability of male condition (compare the light gray areas in figures 6A and 6D with that in figures 6B and 6E). Interestingly, even with low level of direct detectability of male

condition, evolution of male displays becomes feasible under pure epistatic mechanism ($1 < \sigma < 1/s_t$; Figure 6B and 6E). Moreover, when male condition is highly detectable ($\gamma = 0.95$), male displays are more likely to evolve with pure epistatic ($1 < \sigma < 1/s_t$) or partial condition-dependent ($0 < \sigma = \beta < 1$) mechanisms than with revealing ($\sigma = 1$; $\beta = 0$) or completely condition-dependent ($\sigma = 0$; $\beta = 0$) mechanisms (Figure 6C and 6F). Displays that are both epistatic (marginal costs depends upon condition) and revealing (low condition males produce a lower quality display that females do not prefer; $1 < \sigma < 1/s_t$; $\beta = 0$) are the hardest to evolve. The reason that, with highly detectable condition, displays can evolve more easily with pure epistatic or partial condition-dependent mechanisms (or any mechanisms where $\beta > 0$) compared to revealing or complete condition-dependent mechanisms, is that unlike the latter two mechanisms (where $\beta = 0$), males displays can confer an advantage to males even in low condition under the former two mechanisms ($\beta > 0$).

Interestingly, even displays that function completely independently of male condition ($\sigma = \beta = 1$) become easier to evolve when male condition is highly detectable compared to the case of no detectability of condition (compare figures 6A and 6D with figures 6C and 6F). In fact, with very high detectability of condition, completely independent male displays are easier to evolve than revealing indicator displays (Figure 6C and 6F). The reason for this is similar to why pure epistatic indicators become easier to evolve. Male displays that are independent of condition confer mating advantage even when produced by low condition males, and are selected for even with very high detectability of condition; the increase in the frequency of female choosiness with higher condition detectability facilitates the evolution of these displays.

Discussion

Our model shows that the effects of direct detectability of condition on the evolution of female mate choice and on male displays are complex. We find that direct detectability of condition always facilitates evolution of female choosiness, and at certain levels, can actually facilitate evolution of indicator displays. Direct detectability of condition also influences which types of indicator displays are easier to evolve.

Before proceeding with the discussion, we would like to remind the reader that in our model we address the evolution of female choosiness. As discussed in the Introduction, if at least one locus exists that influences female choosiness at a basal level in the nervous system, it would influence female response towards multiple stimuli. Even if female responses towards different male traits are largely independent in a species, as long as there is at least one locus that influences general choosiness in females, the results shown by our model would be qualitatively similar, and still relevant for that species.

Female choosiness

There are two reasons for the facilitative effect of direct detectability of male condition on the evolution of female choosiness. First, direct detectability of condition (γ) reduces the weighted cost of mate choice for females, thus reducing the direct negative selection acting on choosiness. Additionally, direct detectability increases the indirect selection favoring the evolution of female choosiness. A large part of this increase in indirect selection is due to the evolution of a stronger association between female choosiness and high condition (stronger linkage disequilibrium) with increasing direct detectability. The increased direct sexual selection acting on condition, when it is directly detectable, also

contributes to some extent to the increased indirect selection on female choosiness. However, the contribution of higher genetic association is much larger than that of higher sexual selection on condition (results not shown).

Arguments against the evolution of female mate choice through a good genes mechanism often focus on the strength of indirect selection that can cause female mate choice to spread, compared to the strength of direct selection arising through the cost of mate choice. Kirkpatrick and Barton (1997) proposed a framework to measure the strength of indirect selection on female mate choice, with *preliminary* results that indicate that such indirect selection may be too weak to explain the evolution of costly female mate choice. In absence of direct detectability of male condition, the association that builds up between alleles for female mate choice and alleles for condition is usually weak, because it can only build up indirectly through the association between male displays and condition. One effect of direct detectability of condition is that an association between female choosiness and condition can build up without going through the male display. Direct detection of male condition can thus result in stronger indirect selection favoring female mate choice, strong enough to often effectively drive the evolution of choosiness. Studies trying to measure the strength of selection on female mate choice behavior should take into account the possibility of females directly being able to detect male condition.

Male displays

The effect of direct detectability of condition on the evolution of male display is less intuitive than the effect on the evolution of female choosiness discussed in the previous sub-

section. Increasing the direct detectability of condition (γ) essentially reduces how much the male display aids females in the evaluation of male condition. Thus, it reduces the *additional* mating advantage males gain through the display when they already have high condition. Intuition leads to the reasoning that displays should be easier to evolve when they serve as the only clue for females in determining male condition, compared to when they merely aid the evaluation of condition that is already independently detectable to some extent. Yet, we find that at intermediate levels of direct detectability of condition, male displays become easier to spread than in complete absence of direct detectability. The reason for this unintuitive pattern is that increasing γ allows an allele for overall female choosiness to spread to a higher frequency in the population, which increases the overall sexual selection favoring the display. Thus, although the mating advantage with an individual female is weaker, increasing γ increases the number of females that give males with the display a mating advantage. As the detectability of condition increases to very high levels, however, the mating advantage that a mating display provides no longer compensates for the cost of making the display, even with a higher frequency of females that exhibit mate choice favoring male displays. The mating advantage of the display, in such cases, becomes largely restricted to low condition males that produce a display (T_2C_1 males), which does not occur for completely condition-dependent and revealing mechanisms.

Female mate choice favoring certain male displays is thought to evolve because such displays are correlated with some heritable quality in males (Kokko et al. 2003; Andersson and Simmons 2006). Our results suggest that indicator displays are more likely to evolve if the quality that they indicate is partially detectable by females. One aspect of genetic quality that has been shown to be correlated with male mating displays is immune competence

(Kurtz and Sauer 1999; Barber et al. 2001; Ryder and Siva-Jothy 2001; Simmons et al. 2010). An implication of our results is that displays that indicate heritable resistance to diseases with partially visible symptoms may be more likely to evolve than displays that indicate resistance to diseases without visible symptoms or with extremely obvious symptoms. In general, our results imply that indicator displays correlated with disease resistance, body size or other such more perceptible aspects of male genetic quality may be more likely to be found in nature than displays correlated with aspects of genetic quality that are not detectable by females, such as survival rate, growth rate etc. A recent meta-analysis (Prokop et al. 2012) of literature found stronger evidence for indicator traits associated with male qualities such as immune competence and "condition", than for indicators associated with life-history traits. Our results may provide a possible explanation for this pattern.

Continuum

Our model presents a simple framework that encompasses the whole continuum of indicator mechanisms. The three classic indicator mechanisms are based on three types of differences in the displays expressed by high versus low condition males – difference in size (condition-dependent), quality (revealing) and marginal cost (epistatic). These differences, and therefore these mechanisms, need not be discrete or mutually exclusive. We believe that thinking of these mechanisms as discrete cases is unnecessarily restrictive. More importantly, the amount of parametric space that represents the three main mechanisms in isolation is far smaller than the whole possible parametric space, suggesting that indicator displays in most species may function through a combination of these mechanisms. Finally, the continuum framework also allows a direct comparison of different types of indicator mechanisms.

The contribution of a pure epistatic mechanism to the evolution of male displays and female mate choice was long contested, the general consensus now being that pure epistatic indicators are, theoretically, very unlikely to evolve, whereas the evolution of revealing and condition-dependent indicators is theoretically plausible (Pomiankowski 1988; Iwasa et al. 1991; Van Doorn and Weissing 2006). When male condition is not directly detectable by females, our model shows that pure epistatic indicators are harder to evolve than other types of indicators (Figure 6A). With high detectability of condition, however, epistatic indicators become easier to evolve than other types of indicator displays. The reason for this switch in pattern is based on how the mating advantage of the display changes as condition becomes more detectable. As the detectability of condition increases, producing a display confers less and less additional mating advantage to high condition males beyond the advantage conferred by their condition. For completely condition-dependent or revealing indicator mechanisms, the mating advantage of displays is always restricted only to high condition males. These indicator mechanisms therefore are affected more by very high or perfect detectability of condition. For indicator mechanisms in which low condition males also produce a display that confers some mating advantage, such as epistatic indicators and partially condition-dependent indicators, even with very high or perfect detectability of condition, the display can spread in the population due to the advantage it confers to low condition males with displays.

In conclusion, we draw attention to an underappreciated remark by Fisher, highlighted by Maynard Smith (1991). Fisher (1930) stated that females may be able to directly detect heritable differences in male viability, and that female preferences for more viable mates "may therefore be far more widespread than the occurrence of striking

secondary sexual characters." The results of our model emphasize the importance of direct detectability of male condition in shaping the evolution of not only female choosiness, but also male indicator displays.

Proximate mechanisms underlying variation in female choosiness or female mating preferences are not well understood. However, some studies indicate that molecules that influence the nervous system at a basal level may be involved in female mate choice (Appeltants et al. 2002; Vyas et al. 2008; Pawlisch and Riters 2010). These studies only investigate changes in female response towards a single male trait at a time. It would be interesting to see how female responses towards multiple stimuli change with physiological differences in females at such basal level. Understanding how female mating responses towards different male traits function and evolve can shed much light on evolution of reproductive traits of both sexes in general.

Tables

Table 1: Relative viabilities of males of the four expressed genotypes T_1C_1, T_1C_2, T_2C_1, and T_2C_2 are shown. As the H locus is not expressed in males, the values given in this table are identical for males with the H_1 and H_2 alleles. The possible ranges for the viability parameters are $0 < s_t < 1$; $0 < s_c$. The parameter a describes the mating advantage gained by males with choosy females ($0 < a$). Ranges for the two modifier parameters, σ (display cost modifier) and β (mating advantage modifier), are described for each indicator mechanism. The parameter γ describes the degree of detectability of male condition in absence of male display ($0 \leq \gamma \leq 1$). The three classic indicator models in this notation are obtained with $\gamma = 0$.

		Male Viabilities		Female preference for male genotypes	
	Male genotype at T locus / Male genotype at C locus	T_1	T_2	T_1	T_2
General model	C_1	1	$1- \sigma s_t$	1	$1+ \beta a$
	C_2	$1+s_c$	$(1-s_t)(1+s_c)$	$1+ \gamma a$	$1+a$
Completely Condition-dependent $\beta=0$; $\sigma=0$	C_1	1	1	1	1
	C_2	$1+s_c$	$(1-s_t)(1+s_c)$	$1+ \gamma a$	$1+a$
Revealing $\beta=0$; $\sigma=1$	C_1	1	$1-s_t$	1	1
	C_2	$1+s_c$	$(1-s_t)(1+s_c)$	$1+ \gamma a$	$1+a$
Pure epistatic $\beta=1$; $1< \sigma \leq 1/s_t$	C_1	1	$1- \sigma s_t$	1	$1+a$
	C_2	$1+s_c$	$(1-s_t)(1+s_c)$	$1+ \gamma a$	$1+a$

Figure Legend

Figure 1: The continuum of indicator mechanisms is shown as a plane of different combinations of the two parameters σ (modifier of display cost) and β (modifier of mating advantage of display). The three main mechanisms in their purest form lie on the edges or corners of the continuum. A region of biologically unrealistic combinations of Sigma and Beta is shaded out in gray; parametric combinations in this region would indicate low quality males paying a lower marginal cost for creating a display than high condition males. Complete condition-dependence, where low condition males do not produce a display, is shown by a green dot. Partial condition-dependence, where low condition males produce a *smaller* display is shown by dashed green line ($0 < \sigma = \beta < 1$). Values of β lower than 1 indicate a lower likelihood of females mating with T_2C_1 compared to T_2C_2 males, either due to lower quality or smaller size of the display produced by T_2C_1 males. Completely revealing traits are shown by an orange dot, where marginal costs of the display do not differ between males, but females can differentiate between the displays of high versus low quality males and do not prefer T_2C_1 males any more than T_1C_1 males. The blue dot indicates the case where the display is completely independent of condition (neither the expression nor the cost of the display depend on male condition). Values of σ greater than 1 always indicate an epistatic indicator mechanism, because low quality males pay a higher marginal cost for the displays.

Figure 2: The outcome of evolution of female mate choice is shown at different values of direct detectability of condition (γ) and cost of mate choice (s_h), when choosiness and display are initially rare (choosiness frequency $h_2 = 0.05$, display frequency $t_2 = 0.01$). Light gray

region indicates conditions where female choosiness spreads in the population (reaches a frequency greater than 0.999), while dark gray regions show conditions when choosiness is lost (reaches a frequency lower than 0.0001). The three panels show results for the three main indicator mechanisms: condition-dependent indicators (A; $\beta = 0$, $\sigma = 0$), revealing indicators (B; $\beta = 0$, $\sigma = 1$), and pure epistatic indicators (C; $\beta = 1$, $\sigma = 3$). Parameter values used: $a = 3$, $s_t = 0.04$, $s_c = 0.05$.

Figure 3: Changes in frequency of the female choosiness allele h_2 (Δh), when both male display and female choosiness are rare in the population are shown at different values of direct detectability of condition (γ) and cost of mate choice (s_h). Results are shown here for completely condition-dependent indicators ($\beta = 0$, $\sigma = 0$). Results with other types of indicators are visually indistinguishable and are not shown. The values for the components of Δh were calculated by simulating five generations of the life cycle, starting with rare display and choosiness (choosiness frequency $h_2 = 0.05$, display frequency $t_2 = 0.01$, high condition frequency $c_2 = 0.5$) to generate reasonable values of linkage disequilibrium between loci. Parameter values used are: a = 3, $s_t = 0.04$, $s_c = 0.05$. A) Total change in frequency of the female choosiness allele h_2 (due to all direct and indirect selection; equation A5 in the Appendix 2.2). Positive values indicate that the frequency of female choosiness will increase in the population in the current generation, while negative values indicate that it will decrease. B) Change in frequency of h_2 due to indirect selection, which arises through allelic associations (linkage disequilibria) between female choosiness and other loci (equation A6b in the Appendix 2.2). Indirect selection on female mate choice can increase by more than an order of magnitude with increasing direct detectability of condition. C) The primary

component of change in the frequency of h_2 that is due to indirect selection on female choosiness through an allelic association between female choosiness and condition (the quantity $\tilde{a}_C D_{HC}$ in equation A6b in Appendix 2.2). It can be seen that this component accounts for most of the evolution of h_2 due to indirect selection. D) The primary component of change in the frequency of h_2 that is due to indirect selection acting on female choosiness through an allelic association between female choosiness and male display (the quantity $\tilde{a}_T D_{HT}$ in equation A6b in Appendix 2.2). This component, and thus selection through the display locus, accounts for very little of the evolution of h_2 as γ increases. The final term in equation A6b indicates indirect selection on choosiness arising through the association of choosiness alleles with specific *allelic combinations* of the T and C alleles. This represents a component of evolution on h_2 due to indirect selection via both the T and C loci (Barton and Servedio submitted), but the values of this are always extremely small (order of 10^{-6}) and so are not included in the contributions in panels C and D. Parameter values used: a = 3, $\beta = 0$, $\sigma = 0$, $s_t = 0.04$, $s_c = 0.05$.

Figure 4: Evolution of male display at different levels of visibility of condition and display costs, when choosiness and display are initially rare (choosiness frequency = 0.05, display frequency = 0.01). Light grey region – display spreads in the population (reaches a frequency greater than 0.99); dark grey region – display is lost from the population (reaches a frequency lower than 0.0001). A) Condition-dependent indicators ($\beta = 0$, $\sigma = 0$), B) Revealing indicators ($\beta = 0$, $\sigma = 1$), C) Pure epistatic indicators ($\beta = 1$, $\sigma = 3$). Parameter values used: $a = 3$, $s_h = 0.02$, $s_c = 0.05$.

Figure 5: Changes in frequency of the display allele t_2 (Δt) due to direct selection (the net effect of both natural and sexual selection; equation A8a in the Appendix 2.2) acting on the male display when both the display and female choosiness are rare in the population. Methods for calculating Δt are similar to those described for calculating Δh in the legend for figure 3. Parameter values used: : $a = 3$, $s_h = 0.02$, $s_c = 0.05$. A) Condition-dependent mechanism ($\beta = 0$, $\sigma = 0$), B) Revealing mechanism ($\beta = 0$, $\sigma = 1$), C) Pure epistatic mechanism ($\beta = 1$, $\sigma = 3$). Parameter values used: $a = 3$, $s_h = 0.02$, $s_c = 0.05$.

Figure 6: Evolution of male displays with different indicator mechanisms across the continuum of indicator mechanisms (starting choosiness frequency $h_2 = 0.05$, starting display frequency $t_2 = 0.01$). Dark grey region – display is lost from the population (reaches a frequency lower than 0.0001); light gray region – male display spreads in the population (reaches a frequency greater than 0.99). The dotted line is added at $\sigma = 1$, to facilitate easier comparison with figure 1. Panels A through C show display evolution with choosiness set at a higher level ($a = 6$) than panels D through F ($a = 3$). Panels A and D – No direct detectability of condition ($\gamma=0$); panels B and E – Low level of detectability of condition ($\gamma = 0.2$); panels C and F – Very high detectability of condition ($\gamma=0.95$). Other parameter values: $s_t = 0.07$, $s_h = 0.01$, $s_c = 0.05$.

Figures

Figure 1:

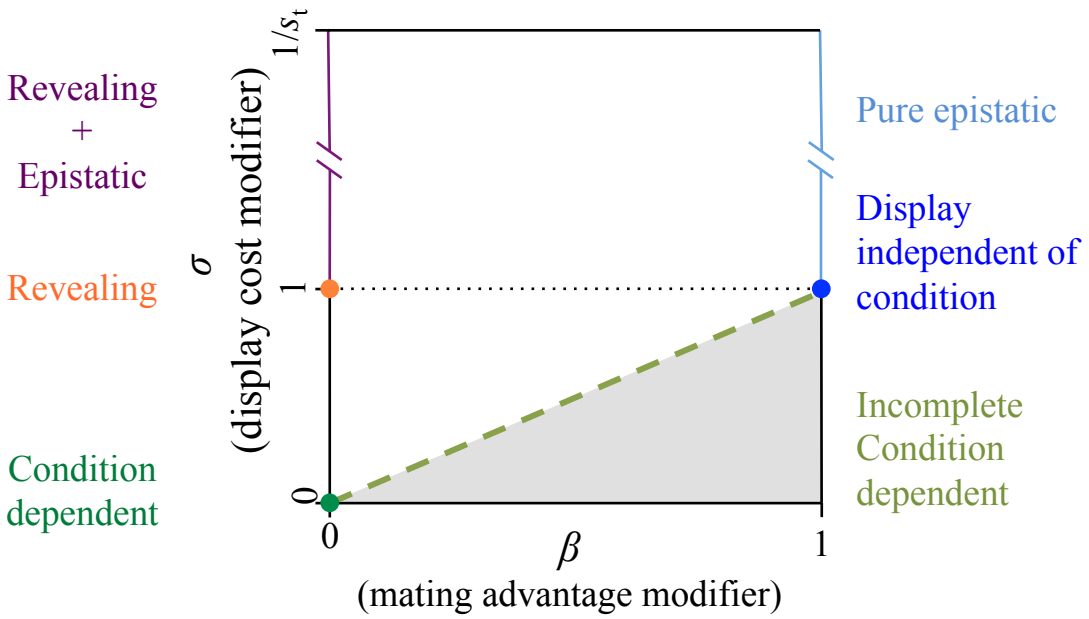


Figure 2:

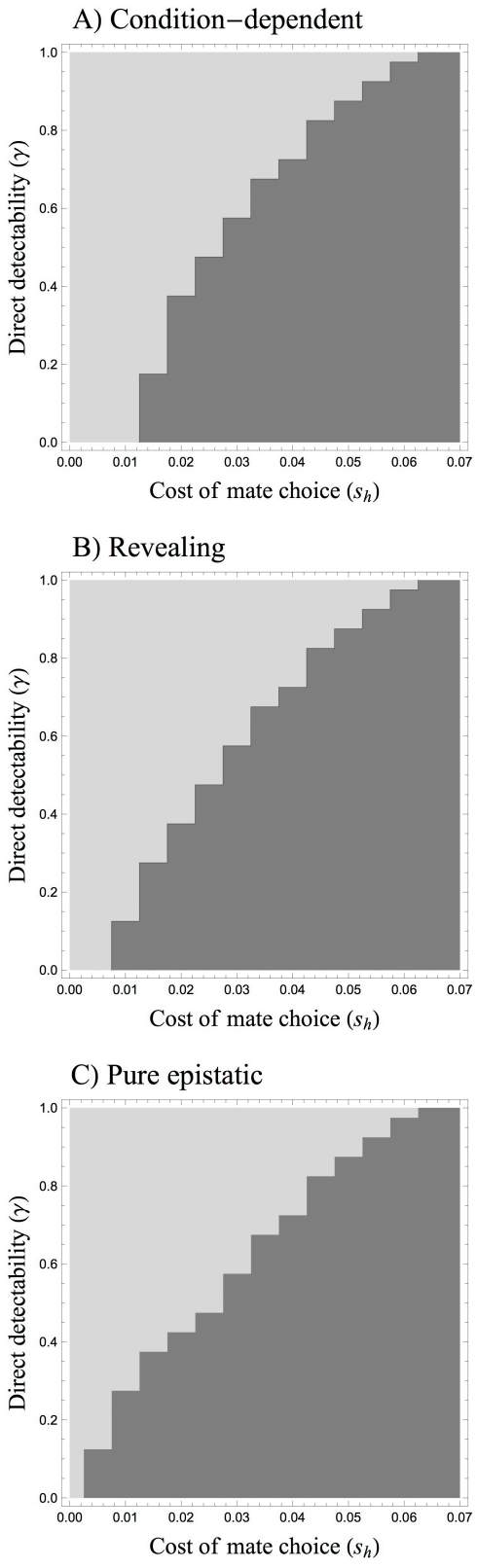

A) Condition−dependent
Direct detectability (γ)
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
Cost of mate choice (s_h)
B) Revealing
Direct detectability (γ)
Cost of mate choice (s_h)
C) Pure epistatic
Direct detectability (γ)
Cost of mate choice (s_h)

Figure 3:

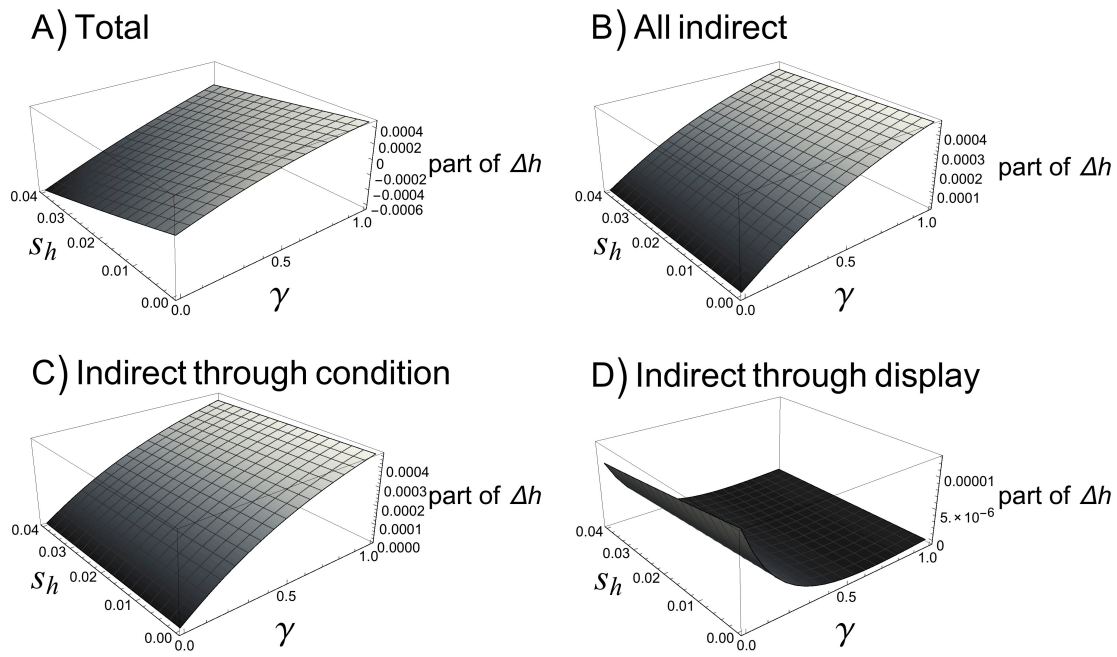

A) Total
0.0004
0.0002
0
−0.0002
−0.0004
−0.0006
part of Δh
0.04
0.03
0.02
0.01
0.00
s_h
0.0
0.5
1.0
γ
B) All indirect
0.0004
0.0003
0.0002
0.0001
part of Δh
C) Indirect through condition
0.0004
0.0003
0.0002
0.0001
0.0000
part of Δh
D) Indirect through display
0.00001
$5. \times 10^{-6}$
0
part of Δh

Figure 4:

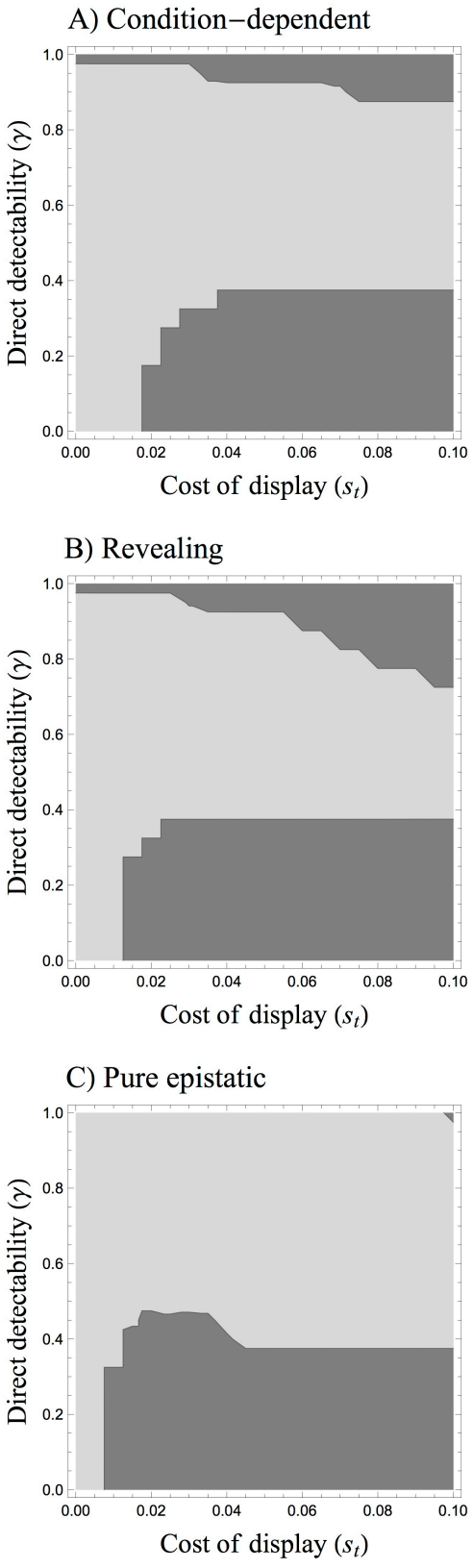

A) Condition−dependent
Direct detectability (γ)
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.02
0.04
0.06
0.08
0.10
Cost of display (s_t)
B) Revealing
Direct detectability (γ)
Cost of display (s_t)
C) Pure epistatic
Direct detectability (γ)
Cost of display (s_t)

Figure 5:

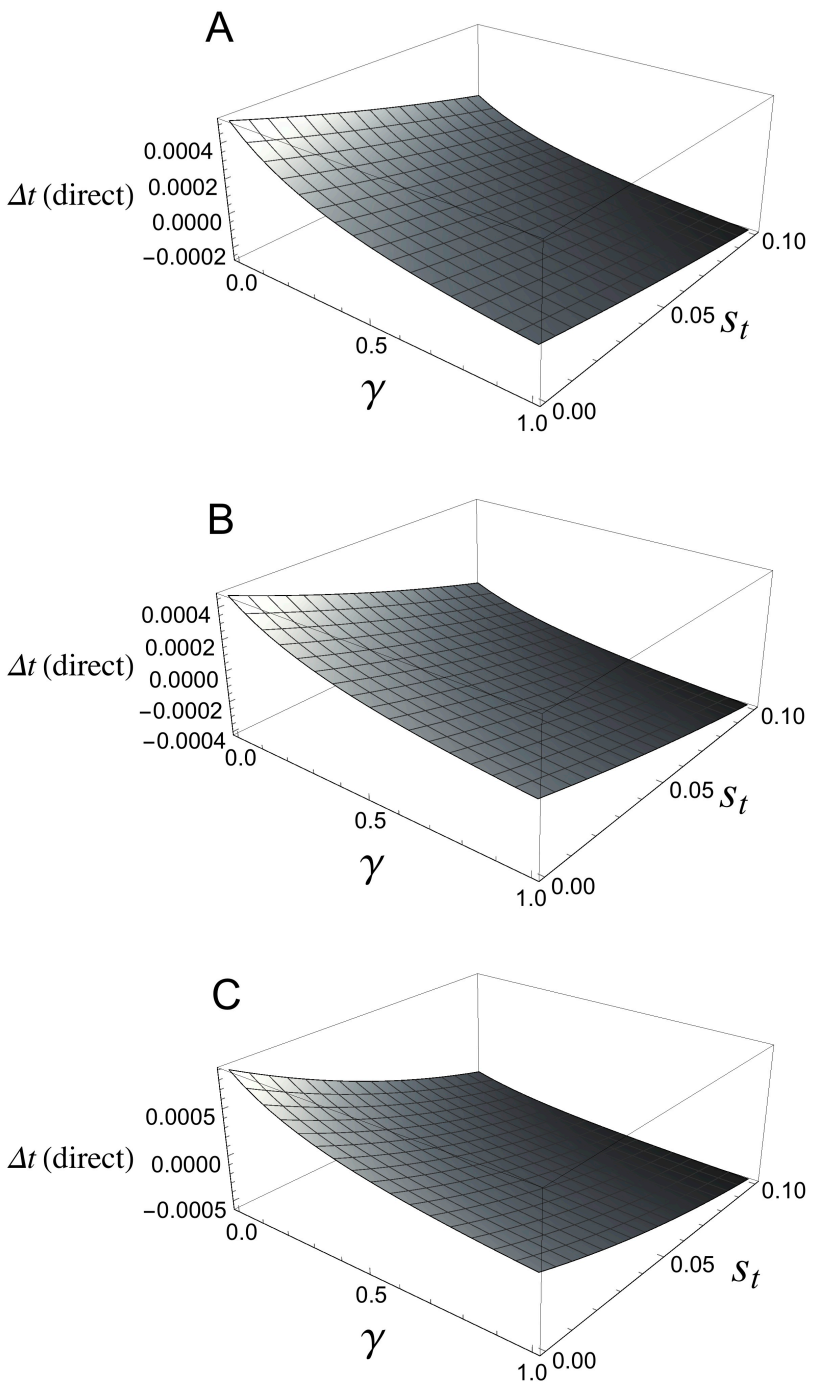
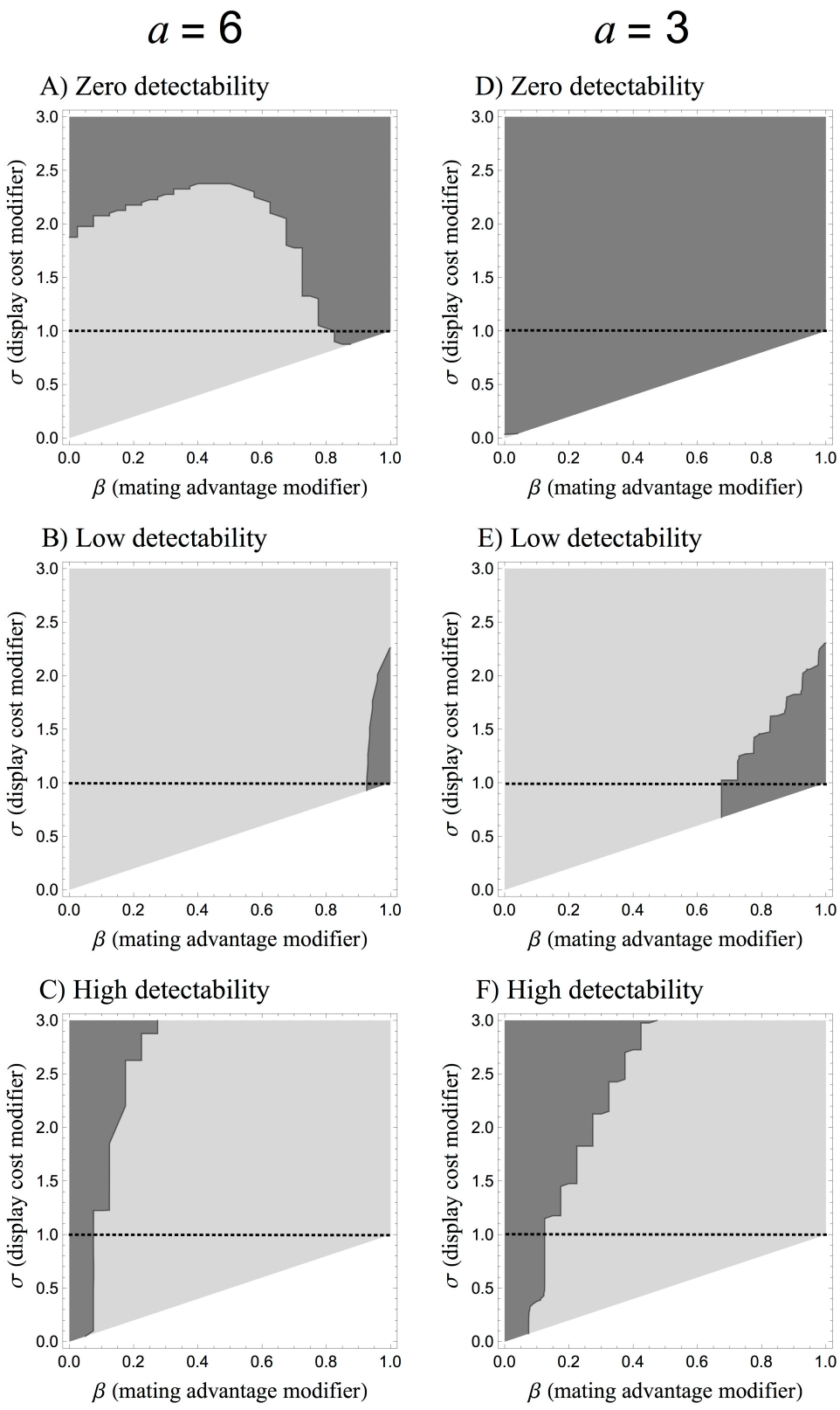

A
Δt (direct)
0.0004
0.0002
0.0000
−0.0002
0.0
0.5
γ
1.0
0.00
0.05
0.10
s_t
B
Δt (direct)
0.0004
0.0002
0.0000
−0.0002
−0.0004
0.0
0.5
γ
1.0
0.00
0.05
0.10
s_t
C
Δt (direct)
0.0005
0.0000
−0.0005
0.0
0.5
γ
1.0
0.00
0.05
0.10
s_t

Figure 6:


a = 6
a = 3
A) Zero detectability
D) Zero detectability
B) Low detectability
E) Low detectability
C) High detectability
F) High detectability
σ (display cost modifier)
β (mating advantage modifier)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.2
0.4
0.6
0.8

CHAPTER III: AGE-DEPENDENT MALE MATING INVESTMENT IN *DROSOPHILA PSEUDOOBSCURA*[1]

Summary

Male mating investment can strongly influence fitness gained from a mating. Yet, male mating investment often changes with age. Life history theory predicts that mating investment should increase with age, and males should become less discriminatory about their mate as they age. Understanding age-dependent changes in male behavior and their effects on fitness is important for understanding how selection acts in age-structured populations. Although the independent effects of male or female age have been studied in many species, how these interact to influence male mating investment and fitness is less well understood. We mated *Drosophila pseudoobscura* males of five different age classes (4-, 8-, 11-, 15-, 19-day old) to either young (4-day) or old (11-day) females, and measured copulation duration and early post-mating fecundity. Along with their independent effects, we found a strong interaction between the effects of male and female ages on male mating investment and fitness from individual matings. Male mating investment increased with male age, but this increase was more prominent in matings with young females. Male *D. pseudoobscura* made smaller investments when mating with old females. The level of such discrimination based on female age, however, also changed with male age. Intermediate aged males were most discriminatory, while the youngest and the oldest males did not discriminate between females of different ages. We also found that larger male mating investments

[1] This chapter is based on Dhole, S. and K. S. Pfennig. 2014. Age-dependent male mating investment in Drosophila pseudoobscura. PLoS One 9:e88700.

resulted in higher fitness payoffs. Our results show that male and female ages interact to form a complex pattern of age-specific male mating investment and fitness.

Introduction

Male reproductive success is generally limited by the number of available mates (Bateman, 1948). Consequently, males are expected to mate indiscriminately so as to maximize the number of matings they obtain (Thornhill and Alcock,1983; Andersson 1994). If, however, mating involves high costs for males (e.g. due to expensive ejaculates), males may discriminate between potential mates by preferring, or providing greater investment in, fitness-enhancing mates (Andersson, 1994; Birkhead and Møller, 1998; Amundsen, 2000; Bondurianski, 2001; Parker and Pizzari, 2010). This discrimination by males is often expressed as facultative adjustments in their mating investment depending on female size, age, or fecundity (Lewis and Iannini, 1995; Martin and Hosken, 2002; Engqvist and Sauer, 2003; Friberg 2006; Cornwallis and Birkhead, 2007; Thomas and Simmons, 2007; Xu and Wang 2009; Lüpold et al., 2011; Simmons 2001; Wedell wt al., 2002). Such context-dependent alteration of mating investment can also depend on the risk of competition with other males, especially the likelihood of sperm competition (Parker and Pizzari, 2010; Parker, 1970; Ingleby et al., 2010; Barbosa, 2011; Sirot et al., 2011; Bretman et al., 2011).

Context-dependent alteration of mating investment can be beneficial for males only if they are likely to mate again (Galvani and Johnstone, 1998; Reinhold et al., 2002). Yet, the chances of future mating opportunities generally decrease with age. Life history theory therefore predicts that males should invest more resources in current matings, and should

become less discriminatory as they age (Galvani and Johnstone, 1998; Reinhold et al., 2002; Gadgil and Bossert, 1970; Stearns, 1976; Michod, 1979; Charlesworth, 1994).

In the same way that a male's age may affect his investment in a given mating, a female's age may also affect her reproductive effort. As females age, they too are expected to invest more of their remaining resources into a given reproductive event as opportunities for future reproduction diminish (Gadgil and Bossert, 1970; Stearns, 1976; Michod, 1979; Charlesworth, 1994). However, because females are often resource limited, the relationship between female age and offspring production is not necessarily straightforward. Indeed, multiple studies have shown that female fecundity generally decreases with age due to senescence (for examples, see Monaghan et al., 2008; Nussey et al., 2008). Regardless of whether female fecundity increases or decreases with female age, any such relationship will generate an effect of female age on her mate's fitness. Consequently, female age is a key feature that might affect male mating investment (for examples, see Xu and Wang 2009; Lüpold et al., 2011; Goshima et al., 1996; Yasui, 1996).

Conversely, because male mating investment strongly influences the reproductive outcome of individual matings (Friberg, 2006; Bretman et al., 2011; Avent et al., 2008; Bretman et al., 2009), male investment will influence not only male, but also female fitness. Thus, changes in mating investment by both males and females as a consequence of their age can influence age-specific fitness (and thus, age-specific reproductive value) of both sexes. Although many studies have examined the effects of either male or female age on mating effort (Martin and Hosken, 2002; Xu and Wang, 2009; Lüpold et al., 2011; Goshima et al., 1996; Avent et al., 2008; Engqvist and Sauer, 2002; Jones and Elgar, 2004), the effects of interactions between male and female age on mating effort—particularly that of males—and

on fitness are largely unexplored. Yet, evaluating the combined effects of male and female age on male mating investment is important, in order to ascertain how age-specific investment in reproduction influences fitness and the evolution of reproductive traits in age-structured populations.

Our goal was to evaluate how males alter mating investment as a function of both their own age and their mate's age. We also evaluated the fitness effects of male investment by assaying female fecundity in response to male investment. As we describe below, we used *Drosophila pseudoobscura* to study the combined effects of male and female age on copulation duration. Copulation duration is often used as a measure of male investment in mating (Dickinson, 1986; Martin and Hosken, 2002; Siva-Jothy and Stutt, 2003; Friberg, 2006; Bretman et al., 2009; Bretman et al., 2010), because it is associated with the size of male ejaculate transferred during mating in a wide variety of insect taxa (Simmons and Siva-Jothy, 1998; Simmons, 2001). We further assayed the fitness effects of copulation duration by measuring the number of eggs females laid following mating.

Methods

Experimental population

The flies for this study came from a laboratory population of *D. pseudoobscura* that was founded in August 2009 with wild-caught flies (10 females and 15 males) collected from Flagstaff, Arizona, USA. All necessary permissions for collection activities were obtained from Flagstaff City Parks department. The study species is not an endangered or protected species, and no additional permissions were required for collection. To establish the population, we mated progeny from all the wild females in a complete reciprocal cross

design (100 mating combinations with three to six replicates). Some of the offspring of each wild female were also mated to at least three of the fifteen wild males. The resulting flies from these matings were then mixed to establish a thoroughly mixed population. This population was maintained with overlapping generations on a cornmeal agar medium under a 12:12 light-dark cycle at 19˚C and 60-80% relative humidity for five and a half months before starting the experiments described below.

To initiate our experiments, we randomly selected 50 flies (25 males and 25 females) from the stock population. These randomly chosen flies were allowed to mate and oviposit in 170ml stock bottles for 6 days. Male and female virgin flies were obtained from the offspring produced in these bottles. We isolated virgin flies within 8 hours from eclosion and housed them individually in 50ml vials, which contained cornmeal agar medium supplemented with yeast granules. Each day, these isolated virgin flies were randomly allocated to the different experimental age treatments so as to avoid any effects of eclosion date.

Mating trials

To evaluate the effects of male and female age on male investment, we used mating trials in which we paired males of different ages with females of different ages and measured copulation duration.

We used copulation duration as our measure of male mating investment, because it is generally correlated with the amount of sperm and/or other components of seminal fluid transferred during mating (Gilchrist and Partridge, 2000; Martin and Hosken, 2002; Avent et al., 2008; Price et al., 2008; Wigby et al., 2009). In *D. melanogaster*, for example, copulation duration is associated with the transfer of seminal fluid proteins (Sfps) (Wigby et al., 2009),

which can have a large effect on male and female fitness (Chapman, 2001; Chapman and Davies, 2004; Ravi Ram and Wolfner, 2007). Indeed, a number of Sfps play a key role in sperm competition (Chapman and Davies, 2004; Wolfner, 1997; Avila et al., 2011). Because copulation duration has these fitness enhancing effects, it is often used as a measure of male investment in individual matings (Martin and Hosken, 2002; Friberg 2006; Monaghan et al., 2008; Bretman et al., 2009, 2010).

To determine the effect of male and female age on copulation duration, virgin females aged 4 days or 11 days were mated to 4-, 8-, 11-, 15- or 19-day old virgin males. We chose these age treatments because they likely represent age classes that are most commonly present in natural populations of *D. pseudoobscura* (Avent et al., 2008; Dobzhanski and Wright, 1943). We did not include older flies, because an estimated 70% of flies die within 14 days from eclosion in the wild (Dobzhanski and Wright, 1943). Male *D. pseudoobscura* usually attain reproductive maturity within 12 hours of eclosion, and carry a full complement of sperm at the age of 2 days (Snook and Markow, 2001). Thus our age classes represented those of sexually mature males that would most likely be exposed to selection in natural populations.

For each mating trial, a single female from one of the two female age treatments was randomly paired with a single male from one of the five male age treatments. To do so, a male was aspirated into the female's vial and he was allowed to mate. In addition to copulation duration, we measured mating latency as the time between introduction of the male into the vial and the start of copulation. We used this mating latency as a measure of female mate preference. Each trial ended when copulation stopped or at 10 minutes if the pair failed to initiate copulation. Virgin *D. pseudoobscura* mate readily, and only two of our

pairings failed to initiate copulation within 10 minutes. We conducted all trials between 2 hours and 6 hours after the lights came on in the incubator. We randomized the order of mating trials on a given day with respect to male and female ages to avoid any order effects, and an equal number of mating trials were conducted on each day for all male-female age combinations. All trials took place in an observation room at 22-25˚C with 50-70% relative humidity. We excluded any matings that were shorter than 60 seconds, because these are likely to be pseudocopulations (Hall, 1978; Barron, 2000). This resulted in the following sample sizes for the female-male age combinations: f4-m4: 16, f4-m8: 11, f4-m11: 8, f4-m15: 10, f4-m19: 10, f11-m4: 10, f11-m8: 10, f11-m11: 10, f11-m15: 7, f11-m19: 7.

In natural populations, older males are unlikely to be virgins. To determine whether any difference in the behavior of older males was a result of age itself or of virginity at old age, we mated a set of 10-day old males to 4-day old virgin females (N=7). The females were discarded, while the males were retained for remating. When these males were 11 days old, they were then mated again to 4-day old females, and the duration of copulation during these matings was measured.

Unfortunately, an incubator failure that resulted in the loss of the study population prevented higher sample sizes in the experiment. Nevertheless, the effects of interest in this study were large enough to be detected with our sample sizes (see Results).

Early post-mating fecundity

Following the mating trials, we measured the effect of copulation duration and male and female age on early post-mating fecundity. To do so, young and old females that had been mated to the youngest, 4-day old, males (N young females = 15, N old females = 9),

intermediate-aged, 11-day old, males (N young females = 7, N old females = 10), and the oldest, 19-day old, males (N young females = 8, N old females = 6) were allowed to oviposit on grape juice-agar medium, supplemented with yeast granules, for two days post copulation. Grape juice-agar medium provided better egg visibility for counting. Because virgin females potentially lay a small number of unfertilized eggs, we collected all eggs and maintained them for 10 days at 19˚C to confirm that they were fertilized (most eggs hatch within three days at 19˚C). Three matings resulted in unfertilized eggs (all three matings were between 4-day old males and 4-day old females). These three matings were excluded from analysis because male fertility could not be ascertained.

Females that did not lay any eggs during the two-day period were monitored for an additional 8 days to determine whether they could produce eggs. Two females failed to lay any eggs during these additional 8 days and were therefore removed from subsequent analyses of fecundity as they were likely infertile. Their removal did not affect the outcome of the analyses. Three females were lost during handling and their fecundity could not be measured.

Statistical analysis

All statistical analyses were performed in R ver. 2.15.1 (R Development Core Team, 2012). The effects of male and female age on mating latency and copulation duration were analyzed using generalized linear models (glm) and a type III ANOVA. Because the copulation duration data showed a quadratic relationship between the variance and the mean (see Fig. S1 in file S1; Table S1 in file S1), a Gamma distribution (with log link function) was used in these analyses. The log link function eliminates meaningless negative time

estimates for copulation duration. Male age and female age were modeled as discrete variables because we used only certain age classes.

The mean-variance relationship of egg laying data grouped by different male-female age combinations revealed that the data were overdispersed compared to a Poisson distribution (Table S2 in file S1). We therefore used the GAMLSS package (Generalized Additive Models for Location, Scale and Shape) in R (Stasinopoulos et al., 2012) to analyze the relationship of early post-mating fecundity with copulation duration, male age and female age. Unlike glm models, gamlss models enable likelihood-based analysis of over-dispersed data. This allowed us to determine which predictor variables and which interactions, if any, generated a model that best fits the data. These log-likelihood tests showed that a negative binomial distribution with a linear mean-variance relationship fit the data better than a Poisson distribution (see Table S3 in file S1). We fit a set of gamlss models in which we evaluated the individual and combined effects of copulation duration, male age and female age on mean early post-mating fecundity. We then used the Akaike Information Criterion with a correction for small sample sizes (AICc) to select the model with the combination of our predictor variables that best explained the variation in early post-mating fecundity of females (Burnham and Anderson, 2002). We note that AICc is an approximate correction for models with non-normal error distribution.

All raw data for copulation duration, latency, egg laying rates and male remating can be found in the supplementary files S2, S3, S4 and S5, respectively that accompany the published version of this chapter (Dhole and Pfennig, 2014).

Results

Mating trials

In our mating trials, we did not find any significant effect of male or female age on latency to mate (Generalized Linear Model, male age:- $t = 1.52$, $p = 0.13$; female age:- $t = 1.24$, $p = 0.22$). Although there was variation in the time it took for males to encounter the females, all males initiated courtship immediately on encountering the female. All females mated within less than 3 seconds after initiation of courtship by any male. Such behavior of virgin females is normal and has been observed in other populations of *D. pseudoobscura* (Noor, 1997; M. Noor, personal communication).

By contrast, copulation duration increased with male age regardless of whether males were mated to young or old females (Fig. 1; ANOVA of glm, $p < 0.0001$; see Appendix 3.1 for detailed model results). The effect of female age was only observed for intermediate-aged males. In particular, 8-day, 11-day and 15-day old males mated for a significantly longer duration with young females than with old females (Fig. 1; Generalized Linear Model; t-values, 8-day: -3.09, 11-day: -3.29, 15-day: -3.80; all p values < 0.003). Consequently, the increase in copulation duration with male age was more pronounced in matings with younger females than with older females (ANOVA of glm with interaction, $p < 0.001$).

Contrary to the findings with the intermediate aged males, copulation duration for the youngest (4-day old) and the oldest (19-day old) males did not differ between females of different ages (Fig. 1; Generalized Linear Model, 4-day old males: $t = 0.42$, $p = 0.67$; 19-day old males: $t = -1.28$, $p = 0.20$)

Copulations of 11-day old males that were previously mated to young females at the age of 10 days (mean = 490 seconds) were significantly longer than the virgin 4-day old

males (mean = 165.5 seconds; glm, $t = 8.857$, $p < 0.01$; Fig. S2 in file S1), but not different from virgin 11-day old males (mean = 477.8 seconds; Generalized Linear Model, $t = 0.184$, $p = 0.855$; Fig. S2 in file S1).

Early post-mating fecundity

We used the AICc and log-likelihood ratios to identify the statistical model that best explained variation in early post-mating fecundity (see Appendix 3.1 for details of model selection and AICc tables). The best fitting model included effects of copulation duration and female age on early post-mating fecundity. A second model with a marginally higher AICc value (but lower AIC value; see Table S4 in file S1) also included significant effects of male age and an interaction between male age and copulation duration on early post-mating fecundity. Given the similarity in AICc values of these two models, and the approximate nature of the AICc correction for models with non-normal errors, we discuss the results of both models. For results that are qualitatively identical for both models, statistics of only the best model are reported here. Detailed output of both models is presented in the Appendix 3.1.

We found that female age significantly affected the number of eggs laid within two days after copulation: young females laid more eggs than old females (Fig. 2; gamlss, $t = -2.57$, $p = 0.013$). We also found that longer copulations resulted in a higher number of eggs laid by the females (gamlss, $t = 3.57$, $p < 0.01$).

According to the second model, male age also affected the number of eggs produced such that females tended to lay more eggs when mated to older males. This pattern was most pronounced for females mated to 11-day old males (who have the longest copulations)

compared to those mated to 4-day old males (who have the shortest copulations; second gamlss model, $t = 2.86$, $p = 0.006$). Females mated to 19-day old males also tended to produce more eggs than females mated to 4-day old males, but the difference was marginal (second gamlss model, $t = 1.97$, $p = 0.055$).

Moreover, according to the second model, a significant interaction exists between the effects of copulation duration and male age on early post-mating fecundity (second gamlss model, 4-day old males vs. 11-day old males: $t = -2.81$, $p = 0.007$; 4-day old males vs. 19-day old males: $t = -2.10$, $p = 0.041$). Copulation duration was positively correlated with the number of eggs laid by females mated to 4-day old males. By contrast, copulation duration did not predict the number of eggs laid by females that had been mated to 11-day and 19-day old males (Fig. 2).

Aside from the uncertainty as to which model best explains early post-mating fecundity, this interaction between male age and copulation duration should be interpreted with caution for two additional reasons. First, the lack of a relationship between copulation duration and the number of eggs produced by females mated to 11- and 19-day old males may be an effect of the small sample size. Second, copulation duration by the youngest males did not overlap with copulation duration by the intermediate-aged or oldest males. Thus, copulation duration and male age are confounded in these groups (see Avent et al., 2008 for a similar effect of male age on copulation duration).

Discussion

We evaluated how male and female age interact to affect copulation duration (our measure of male mating investment) and female fecundity immediately after mating. We

found that investment in mating generally increases with male age, but that intermediate-aged males make the largest investment in mating. We also found that male *D. pseudoobscura* discriminate between females of different ages, and invest more in matings with young females. Moreover, this pattern of mate discrimination coincided with the pattern of mating investment, such that intermediate-aged males displayed the largest difference in investment between young and old females (Fig. 1).

Why should older males copulate for longer than younger males? One explanation is that greater investment in copulation duration by older males represents an evolved response to decreasing chances of future reproduction as they age (Galvani and Johnstone, 1998; Reinhold et al., 2002; Gadgil and Bossert, 1970; Stearns, 1976; Michod, 1979; Charlesworth, 1994). This hypothesis posits that older males will be selectively favored to invest maximally in current matings, because they are less likely to mate in the future. Thus, as males age and opportunities for future reproduction diminish, males should increase investment in any given reproductive bout. Our data were consistent with this prediction. An alternative possibility is that lengthy copulations of older males result from physiological degradation, such that older males require more time to transfer the same amount of resources compared to younger males. However, Avent et al. (2008) found that older *D. pseudoobscura* males transfer higher amounts of sperm during their long matings. Thus, physiological degradation is unlikely to explain our results. We also find that even previously mated intermediate-age males copulate for longer than the youngest males. This result indicates that the increase in copulation duration with age observed in the virgin males cannot be explained solely by the virgin status of 11-day old males, and is instead an effect of male age.

Moreover, male *D. pseudoobscura* increase mating investment when exposed to competitors (Price et al., 2012). Thus, the pattern of increasing mating investment with age may become even more exaggerated in natural populations, because older males are more likely to have been exposed to competitors in nature than younger males.

Another implication of the decreasing opportunities of mating with age is that males should become less discriminatory as they age. As males age, any mating opportunity becomes increasingly likely to be their last. Therefore, old males should invest whatever resources they have in whichever mating opportunity they can find. Thus, although males are expected to invest more in a given mating as they get age, males are also expected to be less discriminatory in their choice of mate with which they make that investment.

Our findings provided mixed support for the notion that males should become less discriminatory with age. At the oldest age class, 19 days, males invested equally in old and young females. That is, copulation duration by males did not differ between old and young females. Because only about 20% of flies are predicted to survive for 19 days in wild populations (Dobzhansky and Wright, 1943), 19-day old males would have very low chances of future matings in the wild. Thus, making a high investment in a mating, regardless of the mate's age, will potentially be selectively favored.

However, contrary to the above hypothesis that males should become less discriminatory as they age, we found that the youngest males did not differ in copulation duration with old versus young females. Indeed, males appeared to become increasingly discriminatory until the last age class (Fig. 1): the difference in copulation duration with old versus young females was most pronounced for intermediate-aged males (Fig. 1). This

pattern suggests that as reproductive investment increased, so too did discrimination up to the oldest age class.

Resource limitation might explain, in part, both the lower overall investment by younger males, and their apparent lack of discrimination among different females. In *D. pseudoobscura*, males do not even begin sperm transfer until about 90 seconds after copulation is initiated (Snook, 1998). The average copulation duration for young males was 169 seconds, which is closer to this minimum time than the average copulation duration for older males with young females (8-day: 336.7s; 11-day: 477.8s; 15-day: 486.5s; 19-day: 405.5s). If young males have not accumulated sufficient resources, they may be restricted to investing in the minimum time required for sperm transfer. By contrast, males aged between 8 and 15 days may have acquired more resources over their lifetime and are therefore able to both engage in longer copulations and differentially invest in copulation duration depending on the female.

Because older females laid fewer eggs than younger females (Fig. 2), males would be expected to invest more in matings with younger females. Our finding that intermediate-aged (8-15-day old) males appeared to be the most discriminating may reflect access to sufficient resources, on the one hand, and sufficient opportunities for future matings on the other hand. By contrast, the youngest males might have inadequate resources to make such differential allocations, whereas the oldest males might be under selection to make maximal investments into a mating regardless of the female because their opportunities for future matings are so low. Generally, males should be most discriminating at intermediate ages when they have both resources *and* opportunities for future reproduction.

From the female's perspective, we found that females laid more eggs following longer copulations. Consequently, females mated to intermediate-aged males laid more eggs than those mated to the youngest or the oldest males. Jones and Elgar (2004) have also found a similar pattern in hide beetles (*Dermestes maculatus*). One possible explanation for this is that females adjust their own reproductive investment depending on either their mate's age or their mate's investment in the mating. However, even *within* the matings with the youngest males females showed higher production of eggs in response to longer copulation times, indicating that the females were not responding solely to male age per se. Thus, females may invest more in matings in which the male also provides enhanced investment.

Moreover, longer copulations may result in the transfer of larger amounts of seminal fluid proteins that stimulate early post-mating fecundity, as occurs in *D. melanogaster* (Wigby et al., 2009). In the absence of direct measures of seminal fluid proteins transferred by the different males, we cannot ascertain whether this possibility accounts for our results. However, given previous findings from *D. melanogaster* (Wigby et al., 2009; Sirot et al., 2009), our results are consistent with this possibility.

The second best statistical model suggests that although females produced more eggs in response to longer copulations with 4-day old males, such a pattern did not arise for the older males (11- and 19-day old). Because there is little to no overlap in duration of copulation by the youngest (4-day old) males and the older (11- and 19-day old) males, this result is difficult to interpret. The result may suggest that copulation duration of older males does not correlate with transfer of seminal fluid proteins that influence egg-laying rates of females. Alternatively, such a pattern could arise if females are unable to increase egg-laying beyond certain physiological limits despite increasing male copulation times. An

experimental resolution between these explanations will require artificial interruption of copulation duration of old males. Such manipulations are associated with their own complications (Gilchrist and Partridge, 2000) and are beyond the scope of this study. Interestingly, in a study on female mating preferences by Avent et al. (2008), young female *D. pseudoobscura* that were mated with old (14-day old) males did not differ from those mated with young (2-day old) males in early post-mating fecundity, but did exhibit higher late post-mating fecundity. The discrepancy between these findings and our results in this study may be a result of differences between populations and between experimental protocols. The population used by Avent et al. had been maintained in the lab environment for 12 to 17 months, and most likely was exposed to strong selection due to high sperm competition (TAR Price, personal communication). Furthermore, males in the experiment by Avent et al. were exposed to competitors prior to mating. Such selection and exposure to competitors may explain the longer copulation durations of their males than males of similar ages in this study. If maximal fecundity stimulation results from high mating investment in all matings, the effects of male investment on early post-mating fecundity may not become apparent.

Our study highlights the way in which differential reproductive investment by males and females can interact to affect the dynamics of both mate discrimination and age-specific fitness from a given mating. These dynamics can ultimately affect the strength and mode of selection in age-structure populations (Charlesworth, 1994). Indeed, age-dependent changes in mating investment will also likely alter the dynamics of sperm competition, and competition itself can alter male mating investment (Parker and Pizzari, 2010; Bretman et al., 2011). Further empirical and theoretical work is needed to better understand these

interactions and their ultimate effects on sexual selection and differentiation among populations that might differ in resource availability, age structure, survivorship, and mate competition.

Figure Legends

Figure 1: The effect of male age on copulation duration with young females (light grey boxes) and old females (dark grey boxes). Asterisks indicate significant differences in copulation duration between old and young females within a male age class.

Figure 2: Effect of copulation duration on the number of eggs laid by females in the first two days after mating. Regression lines obtained from the second gamlss models are plotted. Empty symbols and dashed lines represent matings of 4-day old males; grey symbols and solid grey line depict matings of 11-day old males; filled black symbols and solid black line show matings of 19-day old males.

Figures

Figure 1:

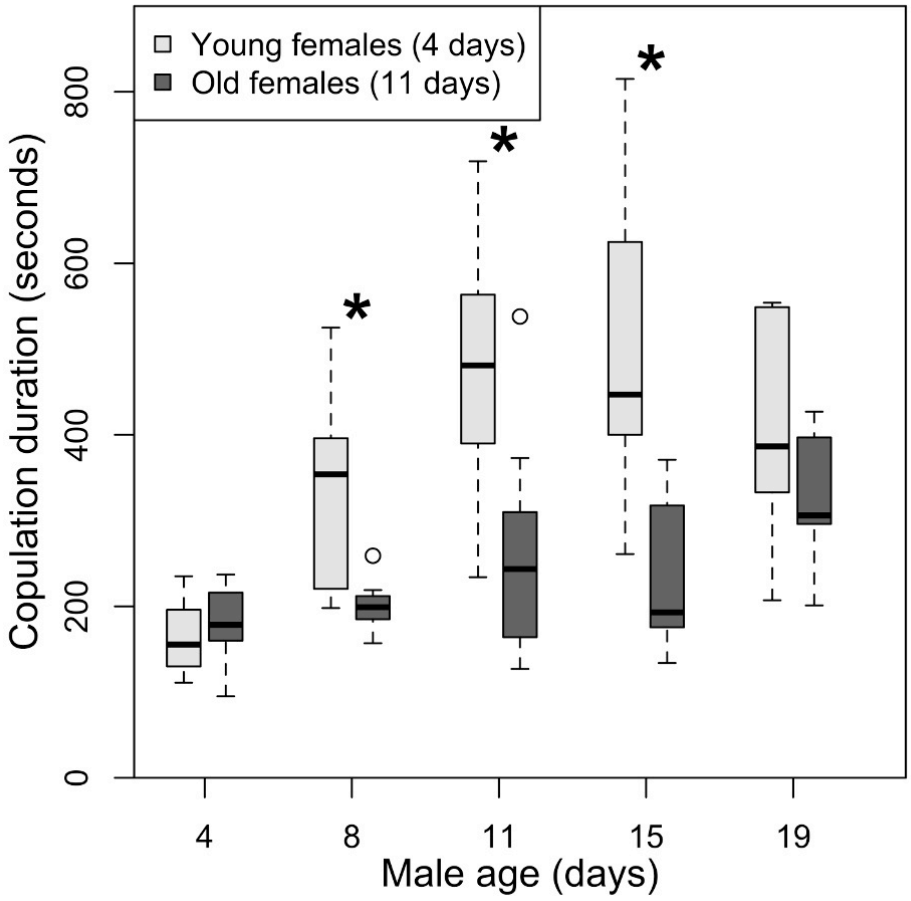
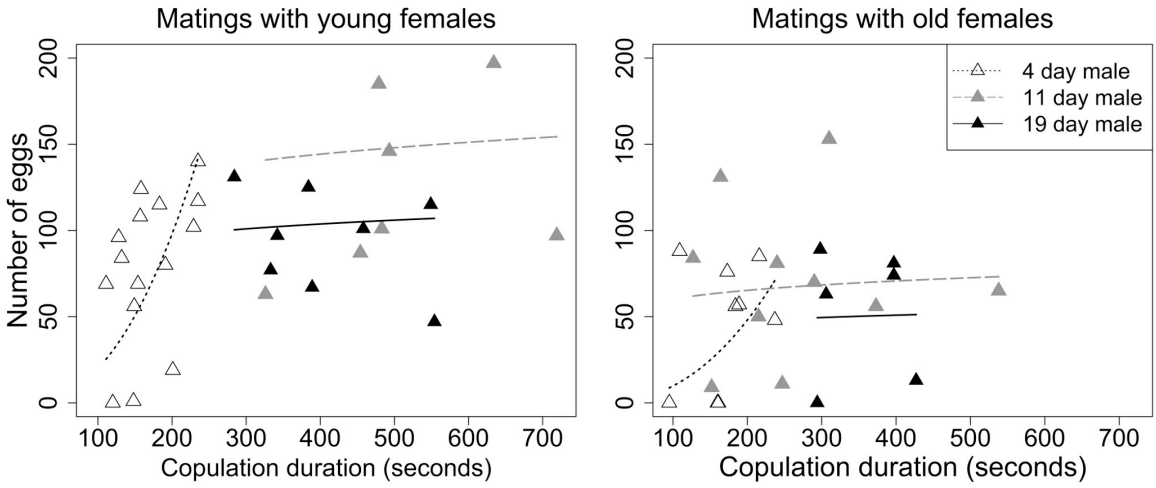


Figure 2:


Matings with young females
Matings with old females
Number of eggs
Copulation duration (seconds)
Copulation duration (seconds)
4 day male
11 day male
19 day male
0
50
100
150
200
100
200
300
400
500
600
700

CHAPTER IV: SPERM COMPETITION AND THE EVOLUTION OF SEMINAL FLUID COMPOSITION[2]

Summary

Male ejaculates include large amounts of seminal fluid proteins (Sfps) that influence male sperm competitive success. In spite of their diverse proximate functions, Sfps involved in sperm competition increase male fitness in one of three ways – 1) "avoidance" proteins help males avoid sperm competition, 2) "defense" proteins help males defend their sperm from displacement by the female's subsequent mate, and 3) "offense" proteins aid males in displacing sperm of preceding males. Here we present a population genetic model of the evolution of allocation of finite resources by males to the three kinds of Sfps. We analyze the influence of relative efficiencies of different Sfps, of plasticity in resource allocation, and of differences in viability costs of Sfps. We find that in absence of plasticity or different viability costs, equal investment in defense and offense Sfps evolves, irrespective of their relative efficiency. In all cases, males evolve to invest more in avoidance when avoidance proteins are increasingly efficient, and when offense is more efficient than defense. Differences in viability costs result in lower investment in costly proteins, while plasticity has complex effects, influencing both the optimal seminal fluid composition and the maintenance of variation in these proteins across populations.

[2] This chapter is based on Dhole, S., and M. R. Servedio. 2014. Sperm Competition and the Evolution of Seminal Fluid Composition. Evolution. 68:3008–3019.

Introduction

Female promiscuity often results in competition for fertilization between sperm from different males (Parker 1970; Birkhead and Møller 1998; Simmons 2001). This competition extends sexual selection beyond the act of mating, and can have a strong effect on male fitness. A male's ejaculate can strongly influence his sperm competitive ability. Hence, a large number of theoretical and empirical studies have addressed the evolution of male allocation of ejaculate towards individual matings, mainly considering the total amount of ejaculate transferred by a male (reviewed in Parker and Pizzari 2010; Enqvist 2012; Parker et al. 2013). A few theoretical studies have also addressed how males should allocate resources towards sperm versus non-sperm components of the ejaculate that stimulate fecundity or bias the outcome of competition between two males (Cameron et al. 2007; Alonzo and Pizzari 2010). However, the evolution of resource allocation among different non-sperm components of the ejaculate has only recently been addressed empirically (Fedorka et al. 2010; Sirot et al. 2011), and has not yet received formal theoretical treatment.

The non-sperm portion of a male's ejaculate generally consists of seminal fluid, which may form a large fraction of his investment in ejaculates (Poiani 2006; Simmons 2001). In humans, for example, seminal fluid constitutes about 95% of the total ejaculate mass (Mortimer 1994). A bulk of the seminal fluid is composed of different seminal fluid proteins (Sfps) in most species (Wolfner 1997; Simmons 2001; Chapman and Davies 2004; Poiani 2006; Findlay et al. 2008), and their production and transfer is often costly (Linklater et al. 2007; Fricke et al. 2008).

The functions of seminal fluid proteins are diverse. They can enhance sperm survival, affect sperm motility, stimulate ovulation and oviposition in females, reduce female

receptivity to mating with other males, or influence competitive interactions between the sperm of rival males (reviewed in Chapman and Davies 2004; Poiani 2006; Ravi Ram and Wolfner 2007). The functions of some Sfps are relevant only in the context of sperm competition; these include proteins that reduce female receptivity to mating, are involved in displacement of rival sperm, or are involved in defending a male's own sperm from displacement by rival males. Furthermore, the benefits of many of these proteins depend upon the mating position of the male (i.e. whether he is a female's first or subsequent mate). Seminal fluid proteins that reduce female receptivity towards rival males only benefit males that mate with females who are in turn likely to mate again, while Sfps that aid in displacement of rival sperm in mated females are useful only for males that mate with non-virgin females. Different Sfps thus affect male fitness at different stages of sperm competition. Because of the diverse functions of different Sfps and the costs associated with their production and transfer (see Linklater et al. 2007), we may expect the evolution of specific patterns of investment by males in different types of Sfps (Perry et al 2013).

Here we use a population genetic model to examine the evolution of male allocation of resources towards Sfps that function at different stages of sperm competition. We focus on the case of females mating with two males, for simplicity. We address the evolution of allocation to Sfps involved in sperm competition that influence male fitness in one of three ways. Seminal fluid proteins that function prior to remating by the female, and thus help males avoid facing sperm competition, are categorized as "avoidance" Sfps (e.g., Sfps that delay remating by the females, or stimulate a female to produce a larger fraction of her progeny before remating; Hartman and Loher 1999; Chapman et al. 2003; Fiumera et al 2005, 2007). We define "defense" Sfps as proteins that function after a second mating to aid

the first male in defending his paternity against the second male (e.g., proteins that reduce sperm displacement by subsequent males; Clark et al 1995; Fiumera et al 2005, 2007). The third category, "offense" Sfps, includes proteins that aid a male mating second out of two males to increase his share of paternity (e.g. proteins that aid in sperm displacement; Harshman and Prout 1994; Prout and Clark 2000; Fiumera et al 2005, 2007). We address the evolution of allocation of a limited amount of resources to proteins that belong to one of the three categories of Sfps. In addition to this basic model, we examine the effects of viability costs of Sfps and of plasticity in Sfp allocation. We find that the optimal seminal fluid composition is determined by the efficiencies of the different types of proteins as well as the level of plasticity and relative viability costs. Specifically, investment in avoidance Sfps increases with the efficiency of offense Sfps relative to defense Sfps, and with the efficiency of avoidance Sfps at delaying female remating. The relative investment in defense and offense Sfps evolves to become equal in absence of plasticity or differences in viability costs. Viability selection has the intuitive effect of reducing investment in costlier proteins. Plasticity in Sfp allocation, on the other hand, has a complex effect on the evolution of seminal fluid composition; it both changes the optimal seminal fluid composition and can allow different populations to reach different compositions based on the initial compositions that the populations possess.

Methods

BASIC MODEL

Our population genetic model with haploid genetics describes evolution at two loci, A and D, that determine how a male allocates his resources to the three categories of Sfps,

avoidance, defense, and offense. We assume that all males have an equal amount of resources. The A locus determines the fraction of resources invested in avoidance Sfps, while the D locus determines how the remaining resources are allocated to the defense and the offense Sfps. Each individual has one of two alleles at each of the loci, designated by the subscripts 0 and 1. Thus our population is composed of the four genotypes A_0D_0, A_0D_1, A_1D_0, and A_1D_1, henceforth referred to as X_1 through X_4 occurring with frequencies x_1 through x_4, respectively. All females mate twice, randomly with respect to the male's seminal fluid composition. A male's fitness is determined by the effects of his Sfps on the fraction of progeny that he sires. We assume non-overlapping generations and even sex ratios.

The life cycle in the model begins with virgin males and females. Males produce seminal fluid as described below. All females mate for the first time with a randomly selected male. The avoidance Sfps transferred during this mating delay remating by the female in proportion to the male's investment in these proteins. Females start producing progeny immediately after the first mating. Higher investment in avoidance Sfps by the first male thus increases the fraction of the female's total progeny that is produced *before* mating with the second male. After the female remates, sperm competition occurs and the remaining fraction of her total progeny is divided between the first and second males as a function of their relative investment in defense and offense proteins respectively. Recombination occurs before progeny are produced.

Seminal fluid production

A male's genotype at the A locus determines the fraction out of his total resources that he allocates towards avoidance Sfps. This fraction is given by the phenotypic value of

the A_i allele, denoted as α_i, which ranges from 0 (no investment in avoidance Sfps) to 1 (all resources go towards avoidance Sfps). The fraction of resources that is not used for avoidance, $(1-\alpha_i)$, is divided into investment in defense and offense proteins based upon the male's genotype at the D locus. A fraction δ_k (corresponding to the phenotypic value of a male's D_k allele) of these remaining resources is invested in defense Sfps, while the remainder $(1-\delta_k)$ is invested in offense Sfps. Phenotypic values (δ) of D locus alleles closer to zero result in lower investment in defense proteins than offense proteins, whereas phenotypic values closer to 1 result in lower investment in offense proteins than defense proteins. The fractions of the *total* resources of a male of genotype A_iD_k that go towards each category of Sfps produced are thus

$$\begin{aligned} &\text{Avoidance investment } (f_A): \alpha_i \\ &\text{Defense investment } (f_D): (1-\alpha_i)\,\delta_k \\ &\text{Offense investment } (f_O): (1-\alpha_i)\,(1-\delta_k) \end{aligned} \qquad \ldots\ldots(1)$$

In nature, seminal fluid protein production may be more likely to be controlled by separate regulatory loci, where the relative amounts of different Sfps in a male's ejaculate are determined by their relative expression levels. This scenario may be better represented by a three-locus model, where each locus determines investment in one of our three categories of Sfps (avoidance, defense and offense). We separately analyzed (numerically) a three-locus version of our basic model, but results from those simulations were qualitatively identical to the results of the two-locus model presented below. The two-locus model is presented here because it allows tractable analytical solutions.

Male sperm-competitive payoffs

All females in the model are assumed to be equally fecund. The portion of a female's total progeny that is produced before the second mating is sired entirely by the first male. This fraction increases with the first male's investment in avoidance Sfps, f_{Ag} for males of genotype X_g, at a rate determined by a scaling parameter σ (where $0 < \sigma < 1$). The parameter σ, which can be interpreted as the efficiency of avoidance Sfps, determines the maximum fraction of progeny that can be secured by a male through a remating delay if he invests all of his resources into avoidance Sfps (i.e., if $f_{Ag}=1$). We first assume that the delay in remating achieved by a male increases linearly with his investment in avoidance Sfps (but relax this assumption below). The progeny gain of a male of genotype X_g through avoidance is thus given by $f_{Ag}\,\sigma$.

The remaining fraction of the female's progeny is divided between the first and the second male based on their relative investments in defense and offense Sfps. When a male of genotype X_g mates first and a male of genotype X_h mates second, the total fraction of the female's progeny gained by the first male is given by

$$m_{gh} = f_{Ag}\sigma + \left(1 - f_{Ag}\sigma\right)\frac{f_{Dg}}{f_{Dg} + f_{Oh}\beta}. \qquad \ldots\ldots(2)$$

In many species one of the two mating positions (mating first or second) confers a sperm-competitive advantage to the male, resulting in first or second male precedence. The parameter β scales the second male's offense investment to determine the relative efficiency of offense and defense proteins. Values of β greater than 1 correspond to offense Sfps being more efficient than defense Sfps (simulating second male precedence), and values of β lower than 1 correspond to defense Sfps being more efficient than offense Sfps (simulating first male precedence). The parameter β in our model is a population-level measure of first or

second male precedence, and does not vary between different males. Examination of alternate forms of payoff from offensive and defensive interactions (e.g., non-linear increases in the effects of proteins with increasing investment) were also analyzed (see Results) but were not found to qualitatively alter the results. The second male, of genotype X_h, sires the remaining fraction of the female's progeny, which is given by (1- m_{gh}).

The total progeny payoff gained by males of genotype *g* competing against males of genotype *h* after mating in both first and second positions with different females is given by

$$\sum_h \left(m_{gh} x_h + (1 - m_{hg}) x_h \right) \qquad \text{.....(3)}$$

The total progeny payoffs of all male genotypes are used to obtain the genotype frequencies in the zygotes, and recursion equations are generated, assuming free recombination. We use the recursion equations for the genotypic frequencies to obtain recursion equations for the allele frequencies at the two loci A and D and the linkage disequilibrium between them. These equations are used to perform invasion analysis to determine the evolutionarily stable seminal fluid composition that evolves in the population under different conditions of first or second male precedence (β) and varying levels of avoidance efficiencies (σ).

Alternative resource allocation structure

In the basic model, the allocation to avoidance Sfps was determined separately by the allele at the A locus, whereas the remaining resources were divided by the D locus into defense and offense Sfps. To confirm that the results of the basic model are not an artifact of this resource allocation structure, we analyzed the two other possible resource allocation structures – 1) Defense allocation determined separately by the D locus, and the remaining resources divided by the A locus into avoidance and offense investments; and 2) Offense

allocation determined separately by the D locus, and the remaining resources divided by the A locus into avoidance and defense investments. We used sequential invasion analysis to determine the optimal seminal fluid composition that would evolve in the population (See Results).

NON-LINEAR EFFECTS OF AVOIDANCE SFPS

In addition to the linear relationship between male investment in avoidance Sfps and remating delay (the fraction of progeny produced before remating) described above, we analyzed the effects of a monotonically increasing concave function and a sigmoidal effect of avoidance proteins in the basic model (Figure 1). Tractable analytical solutions could not be obtained for these non-linear relationships, therefore we used extensive simulations (numerical iterations of the exact recursion equations) to determine the conditions under which an increase (or decrease) in investment in each of the three kinds of Sfps will evolve (see Appendix 4.1).

PLASTICITY

In our basic model, the seminal fluid composition of males is determined by their genotype alone. However, there is evidence that males may display plasticity in their allocation of resources to different Sfps. Males of *Drosophila melanogaster*, for example, can facultatively alter the relative amounts of certain seminal fluid proteins transferred during mating based on whether they are mating with a virgin or a previously mated female (Sirot et al 2011). Both the mechanism by which males make such alterations and the degree to which they can do so are not yet clear. It is possible that plasticity in seminal fluid composition is

more taxonomically widespread. To study the possible effects of plasticity on the evolution of seminal fluid composition, we expand the basic model to allow males to alter their genetically determined seminal fluid composition prior to mating based on the mating status of their mate.

Males mating in the first position do not benefit from the transfer of offense Sfps, whereas males mating in the second position benefit *only* from the transfer of offense Sfps. Therefore, males are expected to reallocate resources from offense proteins towards avoidance and defense proteins when mating in first position, and reallocate resources towards offense proteins when mating in the second position.

We include a parameter μ that determines the level of plasticity in seminal fluid composition. This parameter determines the maximum amount of resources that can be reallocated from one kind of Sfp to others. In nature, the level of plasticity is likely determined by a number of factors, such as the time available to males between determining a female's mating status and copulation, the physiological limits on the rates of protein synthesis (and/or breakdown), and the exact mechanism of alteration.

The genetically determined basal investments in the three kinds of Sfps are given by equation 1. When mating with a virgin female (mating in the first position), males in our model reallocate a maximum of μ resources from their offense proteins to defense and avoidance proteins. Thus, males with a basal offense investment smaller than μ ($f_O \leq \mu$; see equation 1) reallocate all of their offense proteins to defense and avoidance proteins, whereas males with a basal offense investment larger than μ ($f_O > \mu$), reallocate μ resources to avoidance and defense. The reallocated resources are divided between avoidance and defense proteins in proportion to the basal investments in those proteins. The amounts of seminal

fluid proteins transferred after resource reallocation by a male mating in the first position are thus given by

$$\text{Avoidance investment } (f_{A1}):\ f_A + \left(\frac{f_A}{f_A + f_D}\right)\mu\theta + \left(1-\theta\right) f_O \left(\frac{f_A}{f_A + f_D}\right)$$

$$\text{Defense investment } (f_{D1}):\ f_D + \left(\frac{f_D}{f_A + f_D}\right)\mu\theta + \left(1-\theta\right) f_O \left(\frac{f_D}{f_A + f_D}\right) \qquad \ldots\ldots(4)$$

$$\text{Offense investment } (f_{O1}):\ \left(f_O - \mu\right)\theta$$

where $\theta = 1$ if $f_O > \mu$, and $\theta = 0$ if $f_O \le \mu$.

When mating with a previously mated female, males reallocate a maximum of μ resources from avoidance and defense proteins to offense proteins. The amounts of seminal fluid proteins transferred after resource reallocation by a male mating in the second position are thus given by

$$\text{Avoidance investment } (f_{A2}):\ \left(f_A - \mu\frac{f_A}{f_A + f_D}\right)\varepsilon$$

$$\text{Defense investment } (f_{D2}):\ \left(f_D - \mu\frac{f_D}{f_A + f_D}\right)\varepsilon \qquad \ldots\ldots(5)$$

$$\text{Offense investment } (f_{O2}):\ f_O + \left(f_A + f_D\right)\left(1-\varepsilon\right) + \mu\varepsilon$$

where $\varepsilon = 1$ if $\left(f_A + f_D\right) > \mu$, and $\varepsilon = 0$ if $\left(f_A + f_D\right) \le \mu$.

Male mating payoffs are calculated similarly to the basic model, with 'f_{ig}' in equation 2 replaced with f_{i1g} for first mating males (from expression 4), and f_{ih} replaced with f_{i2h} for males mating second (from expression 5). We use invasion analysis to determine the genetically determined evolutionarily stable seminal fluid composition that evolves in the population.

VIABILITY COSTS OF SEMINAL FLUID PROTEINS

In the basic and the plasticity models we assume that investment in different seminal fluid proteins is limited by the finite amount of resources available to males. Increased production of one kind of protein reduces the amount of resources available for the other two kinds of proteins. In addition to such trade-offs, Sfps may incur viability costs on males. We analyze the effect of differential viability costs of the three kinds of Sfps on the evolution of seminal fluid composition. We incorporate a separate viability cost of each kind of proteins – Avoidance cost (c_a), Defense cost (c_d) and Offense cost (c_o). Viability of males with genotype A_iD_k after seminal fluid production is given by

$$v = 1 - c_a f_A - c_d f_D - c_o f_O, \qquad \ldots\ldots(6)$$

where f_A, f_D and f_O are the basal Sfp investments given by equation 1, and c_a, c_d and c_o can range between 0 and 1. In spite of the maximum possible value of 1, we imagine that values for such viability costs in real systems are likely to be very small, so that equation (6) would also approximate multiplicative viability effects. A tractable analytical solution could not be obtained for the model with viability selection. We therefore use simulations to study the effect of differences in viability cost of Sfps on the evolution of seminal fluid composition.

Results

BASIC MODEL

Analytical and numerical analyses of the recursion equations demonstrate that there exist only four mutually exclusive stable monomorphic equilibria. Specifically we can show analytically that when an equilibrium fixed for the genotype A_iD_k is not stable, one of the

other three equilibria (A_iD_l, A_jD_k or A_jD_l fixed) must be stable. Linear stability analysis also shows that no two equilibria can be simultaneously stable. Extensive numerical simulations were also performed, which did not give any indication of the presence of internal (polymorphic) equilibria in the system. Details of these analyses and the analyses below are given in Appendix 4.2.

Relative investment in defense and offense Sfps

We first address the evolution of a male's relative investment in defense versus offense. We find that an allele D_k will increase in frequency and remain fixed in the population, when competing against an allele D_l, provided that

$$\delta_k\left(1-\delta_k\right) > \delta_l\left(1-\delta_l\right). \qquad \ldots\ldots(7)$$

It can be seen from condition (7) that any allele with a phenotypic value δ closer to 0.5 will be selected over an allele with a phenotypic value farther from 0.5. This gives the optimal value for an allele at the D locus $\hat{\delta} = 1/2$. Therefore, given sufficient genetic variation or mutation, alleles that result in a more equal allocation of resources towards defense and offense proteins will replace alleles that cause biased resource allocation. Thus, in the basic case, investment in defense and offense Sfps by males will eventually evolve to become identical.

It is worth noting that condition (7) does not include any terms other than the phenotypic values δ of the two alleles at the D locus. Thus the amount of investment in avoidance proteins does not affect the evolution of the *relative* distribution of the remaining resources to defense and offense Sfps in the basic model. The absolute investments in defense and offense proteins, however, will depend upon the amount of resources not

invested in avoidance by definition. The β parameter, which represents the relative efficiency of defense and offense Sfps, also does not affect the eventual outcome of evolution at the D locus.

Investment in avoidance Sfps

For the basic model, we address evolution at the avoidance locus A primarily as it occurs under the equilibrium values of equal relative investment in defense and offense described above, i.e. when a D_k allele with a phenotypic value (δ_k) of 0.5 is fixed in the population. Specifically, we determine the phenotypic values of A_i alleles that are favored when the D locus has evolved to this equilibrium (see Appendix 4.2). We note that when the alleles at the D locus have not yet reached equilibrium, transient effects on evolution at the A locus can be observed, due to the fact that selection on the alleles at the A locus changes with the frequency of D locus alleles.

In the basic model with linear effects of avoidance Sfps, when locus D is at equilibrium and when defense Sfps are more effective than offense Sfps ($\beta < 1$, potentially reflecting first male precedence), alleles at the A locus with lower phenotypic values (α) are always selected over alleles with higher phenotypic values. Under this scenario, investment in avoidance will decrease, and eventually be eliminated from the population.

In contrast, when offense Sfps are more efficient than defense Sfps ($\beta > 1$, potentially reflecting second male precedence), investment in avoidance Sfps can be maintained if the value of the parameter σ, the efficiency of avoidance Sfps, is sufficiently high. We find that there is an optimal level of investment in avoidance, $\hat{\alpha}$, which will be reached by the

successive fixation of alleles at the A locus that are increasingly closer to this optimum. This optimal value in the basic model is given by

$$\hat{\alpha} = \begin{cases} 0 & \text{if} \quad \sigma \le \dfrac{2}{\beta+1} \\ \dfrac{\sigma(\beta+1)-2}{\sigma(\beta-1)} & \text{if} \quad \sigma > \dfrac{2}{\beta+1} \end{cases} . \qquad \ldots\ldots(8)$$

It can be seen from equation (8) that investment in avoidance, as discussed above, can be maintained only when offense proteins are more efficient than defense proteins ($\beta > 1$), because no value of σ can satisfy the condition for maintaining non-zero investment in avoidance proteins when $\beta < 1$. Even when offense proteins are more efficient than defense proteins, avoidance investment can be maintained only if their efficiency is higher than a critical value that decreases with increasing β. Simulations confirm that an allele at the A locus with the phenotypic value of $\hat{\alpha}$ is favored over any other allele when the corresponding conditions for σ and β are met. Figure 2 shows that the optimal investment in avoidance increases with increasing efficiency of the avoidance Sfps (σ), and with increasing relative efficiency of offense Sfps versus defense Sfps (β). We also re-analyzed the model with two non-linear forms of increase in the effects of offense and defense proteins with increasing investment (see Appendix 4.3), and found that the results above are robust to these modifications. These results are also quantitatively identical to those of the model with alternative resource allocation structures (Appendix 4.4), and thus are not an artifact of the allocation structure used in the basic model.

In sum, with the basic model we find a specific strategy for both investment in avoidance Sfps ($\hat{\alpha}$) and the ratio of investment in defense versus offense Sfps ($\delta_l = 0.5$), that will be reached by successive fixation of alleles at the A and D loci (these represent

convergent stable strategies, CSS, Eshel 1983). Our basic model thus predicts an optimal composition of seminal fluid that is determined by the values of two parameters: 1) the effectiveness of the avoidance proteins in terms of delaying remating (σ), and 2) the relative efficiency of the defense and offense proteins (β).

NON-LINEAR EFFECTS OF AVOIDANCE PROTEINS

In addition to the linear relationship discussed above between male investment in avoidance proteins, f_A, and their effect on the female (the fraction of progeny produced before remating), we address two forms of non-linear relationships (Figure 1). Analytical solutions could not be obtained for these cases. We therefore numerically determined the conditions of σ and β required for any allele that results in a non-zero investment in avoidance Sfps to have higher fitness than an allele that results in no investment in avoidance Sfps. The form of the relationship between avoidance proteins and their effect on the female influences both the maintenance of and the magnitude of investment in avoidance proteins (but not the equilibrium relationship between defense and offense).

When the fraction of a female's progeny that is produced before the second mating increases with diminishing returns with male investment in avoidance proteins (a monotonically increasing concave function), avoidance investment can be maintained in the population under a broader set of conditions than with a linear relationship (Figure 3). Notably, investment in avoidance proteins can be maintained even when offense proteins are less efficient than defense proteins ($\beta < 1$). Moreover, when avoidance investment is maintained, the optimal amount of investment is always higher, across all conditions of σ and β (not shown), than in case of a linear relationship. This is not surprising since with a

concave relationship, avoidance proteins have a higher effective efficiency than with a linear relationship. That is, males always secure a larger fraction of the female's progeny through a given investment in avoidance proteins when there is a concave relationship.

When the effect of avoidance proteins increases in a sigmoidal fashion, evolution of investment in avoidance shows a complex pattern (Figure 3). With a very high efficiency of avoidance (σ), investment in these proteins can be maintained even when offense proteins are less efficient than defense proteins ($\beta < 1$). Thus when σ has high values, a sigmoidal relationship allows the maintenance of investment in avoidance proteins under a broader range of conditions than does a linear relationship. However, as the value of σ decreases, this relationship reverses (Figure 3). Yet, when avoidance investment is maintained, the optimal investment ($\hat{\alpha}$) is always higher in case of a sigmoidal relationship than a linear relationship (not shown).

PLASTICITY

Inclusion of plasticity in Sfp investment has a complex set of effects on the evolution of seminal fluid composition. Recall that in our models without plasticity we find a single convergent-stable combination of allelic values ($\hat{\alpha}$ and $\hat{\delta}$) at the two loci, resulting in a single optimal seminal fluid composition that evolves in the population with a given set of parameter values (σ and β). With plasticity in male seminal fluid investment, we find that multiple combinations of allelic values (α and δ) that can be evolutionarily stable when invading alleles are rare (Figure 4; Appendix 4.5). These combinations of allelic values form the curve of evolutionary stable seminal fluid compositions that a population can reach and remain at. The stable seminal fluid composition that will ultimately be reached by a specific

population depends upon the initial seminal fluid composition in that population. This means that different populations can evolve different stable seminal fluid compositions under the same parameter values, depending on the initial genetic variation (or sequence of new mutations) in those populations (different starting locations on the planes in Figure 4A). Furthermore, unlike in the basic model, when males can plastically alter their seminal fluid composition investment in avoidance proteins can be maintained even when defense proteins are more efficient than offense proteins ($\beta < 1$; simulating first male precedence; Figure 4B).

Additionally, we find that the level of plasticity (μ) can strongly influence the identity of these evolutionarily stable seminal fluid compositions. At very high levels of plasticity, males evolve to invest only in avoidance and offense proteins (Figure 5). At low levels of plasticity, investment in all three categories of proteins can be maintained.

Inclusion of plasticity in Sfp allocation also allows evolution of unequal basal investment in offense and defense proteins, which otherwise always evolve to become equal. In fact, the basal investment in offense Sfps more often evolves to be higher than that in defense Sfps than vice versa (ESS values of δ are mostly smaller than 0.5; Figures 4, 5, 6). The effects of the relative efficiency of defense versus offense proteins (β) and of the efficiency of avoidance proteins (σ) on the evolution of basal avoidance investment remain qualitatively similar to our basic model – basal investment in avoidance increases with higher β and with higher σ.

VIABILITY COSTS OF SFPS

When seminal fluid proteins incur a viability cost in addition to trading off with other Sfps, changing the cost of a seminal fluid protein has an intuitive effect on investment in that

protein – males evolve to make smaller investments in Sfps that are more costly. Unequal costs of offense and defense proteins ($c_d \neq c_o$) can thus result in the evolution of unequal investment in these proteins ($\hat{\delta} \neq 0.5$; Figure S2 in Appendix 4.6). The cost of avoidance proteins has an effect analogous (although opposite in direction) to the efficiency of avoidance proteins (σ). As the cost of avoidance proteins (c_a) increases, populations evolve to be fixed for lower investment in avoidance proteins; and as the advantage to the second male (β) increases, higher avoidance investment can be maintained for a given cost of those proteins (Figure S3 in Appendix 4.6).

Discussion

We examine the relative investment that males will evolve to make in seminal fluid proteins (Sfps) that perform avoidance, defense or offense functions during sperm competition. We find that in the absence of plasticity and differences in viability costs males will evolve to invest equally in defense and offense proteins, independent of their relative efficiency. However, with plasticity in seminal fluid composition or with unequal viability costs of Sfps, the relative investments in defense and offense Sfps can evolve to become unequal. We also show that the optimal investment in avoidance proteins increases with both the relative efficiency of offense versus defense and the efficiency of the avoidance proteins themselves.

Investment in defense and offense sfps

The evolution of equal investment in defense and offense proteins in the basic model is a consequence of the payoff ratios that emerge under random mating. When mating is

random, all male genotypes are equally likely to mate first or second. Furthermore, we assume that a trade-off exists between investing resources in different Sfps, so an increase in investment in one type of protein results in a decrease in the investment in others. Under these conditions it can be seen that the increased payoff in, for example, the second position due to increased offense investment does not compensate for the decreased payoff in the first position due to lower defense investment (for example, see Figure 6A). Thus splitting resources equally between offense and defense proteins maximizes the total payoffs for mating across both the first and second positions. Our basic model also predicts that, when males are equally likely to mate in the first or second position, the evolution of relative investment in defense and offense Sfps is not influenced by the relative efficiency of these two types of proteins (β). Any advantage that males gain in one mating position by skewing their allocation towards the more efficient proteins, is again compensated by a disadvantage in the other position. Just as when offense and defense are equally efficient, when $\beta>1$ (offense Sfps more efficient), a male investing more in offense benefits when mating in the second position, but suffers a high paternity loss when mating in the first position, because the competing male has the offense advantage (see Figure 6B). Analogous results have been found in models of sperm competition that have studied investment in sperm number by males that mate first or second, when one of the mating positions confers an advantage (e.g. Parker 1990; reviewed in Parker and Pizzari 2010). That is, when mating positions are determined randomly, and competition functions as a "loaded raffle" (Parker 1990; similar to the interaction between defense and offense Sfps here), optimal sperm investment is identical for a male mating in the first or second position irrespective of the level of unfairness of the raffle (Parker 1990).

Unlike in the basic model, when males can plastically alter their seminal fluid composition based on their mating position (first or second), we find that unequal investment in defense and offense proteins can easily evolve, generally with higher basal investment in offense than defense Sfps (evolutionarily stable values of δ are generally < 0.5; Figure 4). When mating in the first position, males reallocate their offense investment towards avoidance and defense, whereas when mating in the second position, they reallocate avoidance and defense resources towards offense. The set of evolutionarily stable seminal fluid compositions is restricted to basal investments that allow males to reallocate the maximum amount of resources possible (given by the level of plasticity, μ) in both first and second mating positions. This is possible only when the basal investment in the proteins being reallocated is larger than or equal to μ in both mating positions, i.e. when $f_{\mathrm{O}} \geq \mu$ and $\left(f_{\mathrm{A}} + f_{\mathrm{D}}\right) \geq \mu$. This is why when there is a significant investment in avoidance (f_{A}), the evolutionarily stable allelic values for the D locus (δ) are generally lower than 0.5; values of δ greater than 0.5 would not allow the condition $f_{\mathrm{O}} \geq \mu$ to be satisfied when there is significant investment in avoidance.

Another factor that may result in the evolution of unequal investment in defense and offense proteins in natural populations is a bias in mating order. In nature mating order biases can exist for many reasons, e.g. male dominance rank may determine the order of mating, or female mating preferences may change with her mating status (e.g. Richards 1985; Teuschl and Blanckenhorn 2007). If certain males are more likely to mate with virgin females versus mated females, we expect that those males would evolve to make higher relative investments in defense and avoidance, while males that are more likely to mate with non-virgin females would evolve to invest more in offense.

Investment in avoidance sfps

In all our models we find that the relative efficiency of defense and offense proteins influences the evolution of investment in avoidance proteins. Specifically, for any investment in avoidance to be maintained in a population, avoidance proteins need to have a certain minimum efficiency (σ) for a given relative efficiency of offense versus defense proteins (β). If avoidance proteins are less efficient than this critical value, males evolve to invest nothing in avoidance proteins. This minimum level of avoidance efficiency decreases with increasing β in all models, indicating that increasing offense efficiency can allow investment in less efficient avoidance proteins to persist.

With a given relative efficiency of defense and offense proteins, the minimum required level of avoidance efficiency differs among our model variants. In the basic model, when defense is more efficient than offense ($\beta < 1$, first male sperm precedence) avoidance cannot evolve at all. In contrast, in the versions of our model with non-linear avoidance effects, plasticity, or viability selection, investment in avoidance proteins can evolve in spite of more efficient defense than offense. Because biological molecules often have non-linear dose-dependent effects (Wright 1934; Kacser and Burns 1981), it is very likely that in most species avoidance proteins have a non-linear, plateauing effect on females (Figure 1), as opposed to the linear relationship assumed in our basic model. In order for investment in avoidance evolve the efficiency of avoidance proteins however needs to be much higher when defense is more efficient compared to cases with more efficient offense. These results suggest that investment in avoidance can exist, but may be less common, in species that exhibit first male sperm precedence compared to species with second male precedence.

When investment in avoidance does evolve, our results predict that it will reach a specific optimal value, or one out of a set of optimal values in the case where there is plasticity. For the basic model this value is given by $\hat{\alpha}$ (Equation 8). We find that males will ultimately evolve to invest a larger fraction of their resources in avoidance proteins as the efficiency of offense versus defense proteins increases (greater values of β). In natural systems, a male would benefit more by avoiding direct sperm competition when his competitor is expected to have an advantage over him. This implies that males from species with stronger second male precedence are likely to invest more resources in avoidance Sfps. We find that males also evolve to invest more in avoidance Sfps as the efficiency of these proteins (σ) increases; with higher efficiency of avoidance proteins males gain higher fitness in the first position through a given amount of investment in avoidance. Interestingly, in all models that we examine the optimal investment in avoidance Sfps comprises a larger fraction of the total resources available to a male than the investment in either offense or defense proteins. This is apparent from our finding that when males evolve to invest in avoidance proteins, they often evolve to invest more than $1/3^{rd}$ of their resources in avoidance (optimal values for α are often > 0.33; Figures 2, 4, 5). Avoidance Sfps are thus more likely to form a larger chunk of the seminal fluid than either defense or offense Sfps.

Plasticity and multiple evolutionarily stable compositions

In absence of plasticity we find a single optimal allelic value each for the two loci (single optimal seminal fluid composition) that will evolve by successive fixation of alleles with values closer and closer to these optimal values. A population thus approaches the same optimal seminal fluid composition irrespective of the initial distribution of alleles in the

population (a globally convergent stable strategy – CSS). With plasticity in seminal fluid composition, we find that the optimal seminal fluid composition is frequency dependent. That is, we find the existence of multiple compositions that are optimal when at high frequency and cannot be replaced by new rare compositions (evolutionarily stable strategies – ESSs), but do not necessarily increase in a population when at low frequency. With plasticity, a population fixed for one of the optimal seminal fluid composition can evolve to be fixed for another seminal fluid composition, if there is a large influx of alleles for the new composition. Such an influx could occur in natural populations due to migration from neighboring populations that may be fixed for a different optimal seminal fluid composition. Even in absence of genetic variation at the two loci, plasticity in resource allocation allows males from a population to transfer different seminal fluid compositions based on the mating position that they occupy, maintaining phenotypic diversity in the population. The presence of multiple evolutionarily stable compositions suggests that plasticity can also facilitate the maintenance of genetic variation across populations – different populations can evolve to be fixed for different seminal fluid compositions, even if they do not differ in any other way besides initial genetic variation in seminal fluid composition.

Due to the methodological limitations on measuring fine-scale changes in seminal fluid proteins, currently evidence for plastic alteration of the relative amounts of Sfps in the ejaculate is available only from *Drosophila melanogaster* (Sirot et al 2011). Furthermore, the exact mechanism of this plasticity is not yet clear. A number of mechanisms are possible. For example, males may break down existing proteins and use those resources to synthesize new ones, they may store free resources uncommitted to any Sfps that are facultatively used for protein synthesis upon encountering a female, or they may simply transfer differing amounts

of proteins that they already possess. Our model of plasticity can represent the first of the above possible mechanisms over the whole parametric space, and also the second mechanism when μ is smaller than the basal investments in Sfps ($f_O > \mu$ and $\left(f_A + f_D\right) > \mu$). The exact mechanism of plasticity is likely to influence the type of trade-offs between investing in different Sfps, and in turn the effect plasticity will have on the evolution of seminal fluid composition.

To some extent, the complexity of the results of our model with plasticity arises from the way plasticity is incorporated. The level of plasticity (μ) in our model sets an *absolute* maximum limit on the amount of resources that males can reallocate before mating. This contrasts with setting a *proportion* of basal investment that is reallocated. Modeling plasticity by allowing μ to set a proportion would most likely make some of the discrete effects of our parameters more continuous. But we believe that an absolute limit on the reallocation is biologically more realistic. Reallocation of resources from one type of protein to another is most likely limited by rates of physiological processes such as protein synthesis and breakdown. These rates are likely to limit the maximum protein turnover possible in a given amount of time, instead of determining a proportion of the basal investment that is reallocated. The complexity of our results may be a reflection of the complex nature of the physiological process of resource reallocation.

Although we do not incorporate any coevolutionary dynamics between males and females in our model, our results have interesting implications regarding the effects of sexual conflict on male seminal fluid composition. Sexual conflict can arise when the fitness interests of males and females diverge (Parker 1979; Arnqvist and Rowe 2005). It is in a male's interest to prevent, or at least delay, his mate's subsequent mating with another male

(or likewise to cause his mate to produce a larger fraction of her total progeny before mating with another male). Males are therefore expected to evolve increasingly efficient avoidance proteins (Rice and Holland 1997). Females, on the other hand, may benefit by mating with other males, e.g. by getting better or more varied sperm, by gaining resources, or to avoid sperm limitation (Thornhill and Alcock 1983; Arnqvist and Nilsson 2000; Jennions and Petrie 2000). Females are therefore expected to evolve resistance to males' avoidance proteins. Indeed, such an evolutionary arms race between males and females is thought to be one of the reasons for the rapid evolution of some of the Sfps that delay female remating or increase female egg-laying rates (Rice and Holland 1997). The σ parameter in our model describes the efficiency of male avoidance proteins. Selection on males to delay remating would have the evolutionary effect of increasing the value of σ, while the evolution of female resistance to male avoidance proteins would reduce the value of σ. Interestingly, our model predicts that when females evolve resistance to male avoidance proteins, males will evolve to invest fewer, not more, resources in avoidance proteins (lower σ leads to lower $\hat{\alpha}$). This is because as females become resistant to male avoidance Sfps, these proteins become less and less worth investing resources in. Males would benefit in these cases by allocating those resources to other types of Sfps.

When sexual conflict occurs, females also may benefit by controlling the advantage to their first or second mate, thus influencing β. For instance, females may benefit by increasing second male precedence (greater β) if their second mate is more attractive. Our results predict that this will lead to a greater investment by males in avoidance proteins.

Our goal in this study was to design a model that could yield some basic predictions about the evolution of seminal fluid composition, and serve as a building block for more

complex and system-specific models. The functions of a large number of seminal fluid proteins still remain unknown. However, we do know the functions of some seminal fluid proteins, especially in insects, which may thus be a good basis for future study (reviewed in Chapman and Davies 2004; Poiani 2006; Ravi Ram and Wolfner 2007). Systems like *Drosophila* may prove useful for testing some of the predictions of our model through artificial selection experiments that control factors such as mating order, the delay in remating, and, to some extent, the levels of advantage to the first or second male.

Figure legends

Figure 1: Three different relationships between male investment in avoidance proteins and the fraction of a female's progeny that is produced before remating. The net slope and the maximum value reached by each function is determined by σ. Shown here are functions with $\sigma = 1$. Solid line: a linear relationship, dashed line: a concave relationship, dotted line: a sigmoidal relationship.

Figure 2: Optimal investment in avoidance proteins, $\hat{\alpha}$, as a function of the efficiency of avoidance proteins (σ) and the relative efficiency of offense versus defense proteins (β).

Figure 3: Conditions for maintenance of non-zero investment in avoidance proteins (shaded area above curves) with linear (solid line), concave (dashed line) and sigmoidal (dotted line) relationships between investment in avoidance proteins and the fraction of progeny produced before female remating.

Figure 4: Curves of critical values are shown for evolution at the two loci (thin dashed line – D locus; thin solid line – A locus; thick solid line and black dot – overlapping parts of the curves for the two loci). When a population is fixed for allelic combinations that lie in the area enclosed below (along the *y*-axis) the curve of critical points for a given locus, alleles at that locus with slightly higher values than the resident allele increase in frequency, whereas above the curves alleles with smaller values increase in frequency (note that there is an exception to these patterns in the area enclosed by the small second curve for the A locus in

the lower left of Figure 4B). The arrows depict the direction a population will evolve along, given successive fixation of mutations with small effects. Arrows are *not* drawn to scale with the rate of evolution. The thick solid line shows evolutionarily stable combinations of allelic values. A) $\mu = 0.35$; $\beta = 2$; $\sigma = 0.4$; B) $\mu = 0.35$; $\beta = 0.6$; $\sigma = 0.7$. Details of how these figures were generated are given in Appendix 4.5.

Figure 5: Joint critical curves depicting the evolutionary stable seminal fluid compositions are shown for different levels of plasticity (μ). Dark gray solid curve and dot: $\mu = 0.25$; Black solid curve and dot: $\mu = 0.35$; Light Gray dashed curve: $\mu = 0.45$; Black dashed line: $\mu = 0.55$; Dark gray dotted line: $\mu = 0.65$; For all curves $\beta = 2$; $\sigma = 0.4$.

Figure 6: Relative investments in offense (f_O, dark gray sectors) and defense (f_D, light gray sectors) proteins of competing males, *i* (on the left side of black vertical line) and *j* (on the right side of black vertical line), bearing an allele at the D locus with value δ_1 *or* δ_2. Relative sector angle indicates relative investment in a given Sfp. Males with the allele for δ_1 invest equally in offense and defense proteins, while males with the allele for δ_2 invest three times as much in offense as defense. Progeny shares gained through defense (when mating 1st) and offense (when mating 2nd) by *j* males (with either δ_1 or δ_2) are calculated when they compete with a male *i* (with δ_1). Checkered colors show competing Sfps when male *j* mates first, while plain colors show competing Sfps when male *j* mates second. A) A case with equally efficient defense and offense. B) Offense proteins are twice as efficient as defense proteins. Larger radii of investment sectors indicate more efficient proteins (not drawn to scale). Males with equal investment in offense and defense proteins have higher total payoff than males

with unequal investment in offense and defense irrespective of the relative efficiency of the two kinds of proteins.

Figures

Figure 1:

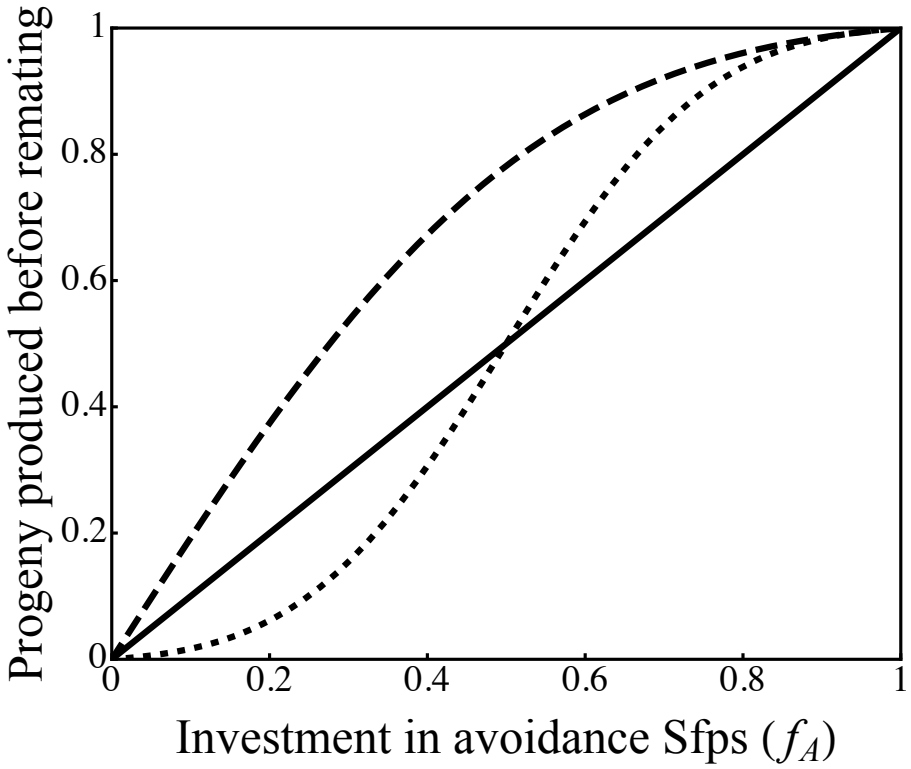


Figure 2:

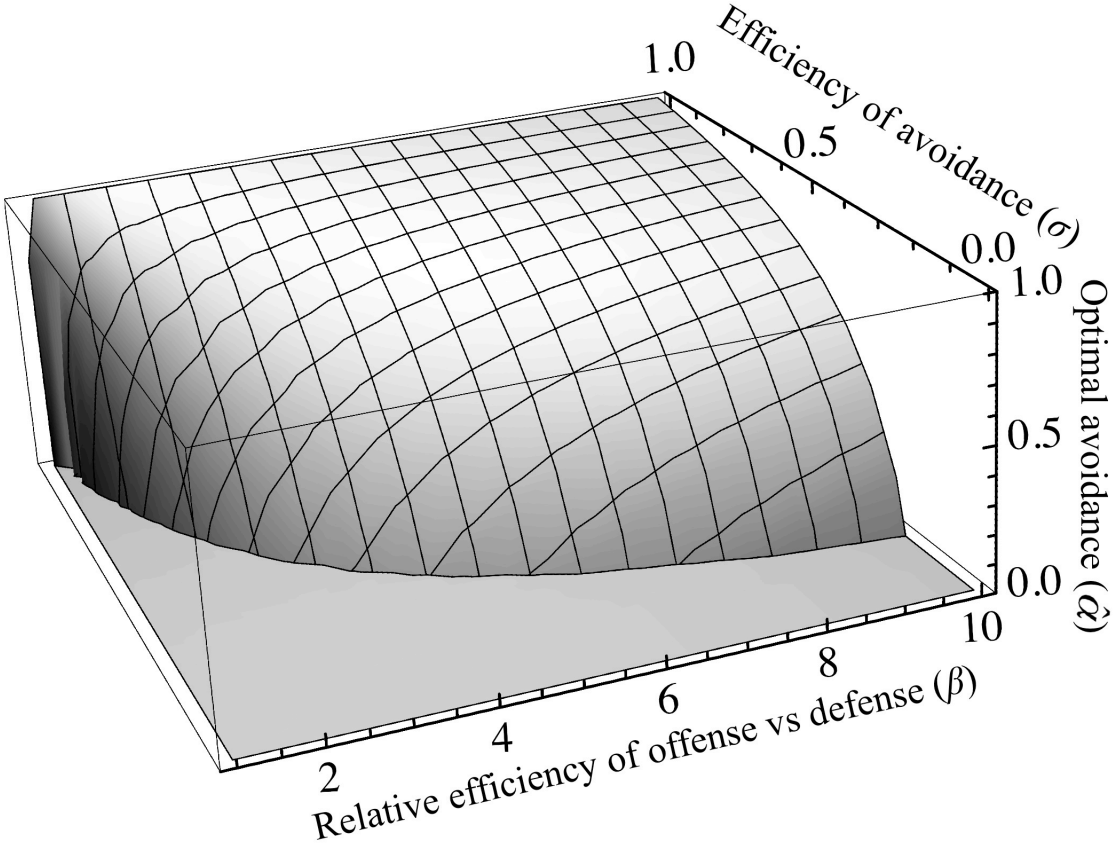

Efficiency of avoidance (σ)
1.0
0.5
0.0
Optimal avoidance ($\hat{\alpha}$)
1.0
0.5
0.0
Relative efficiency of offense vs defense (β)
2
4
6
8
10

Figure 3:

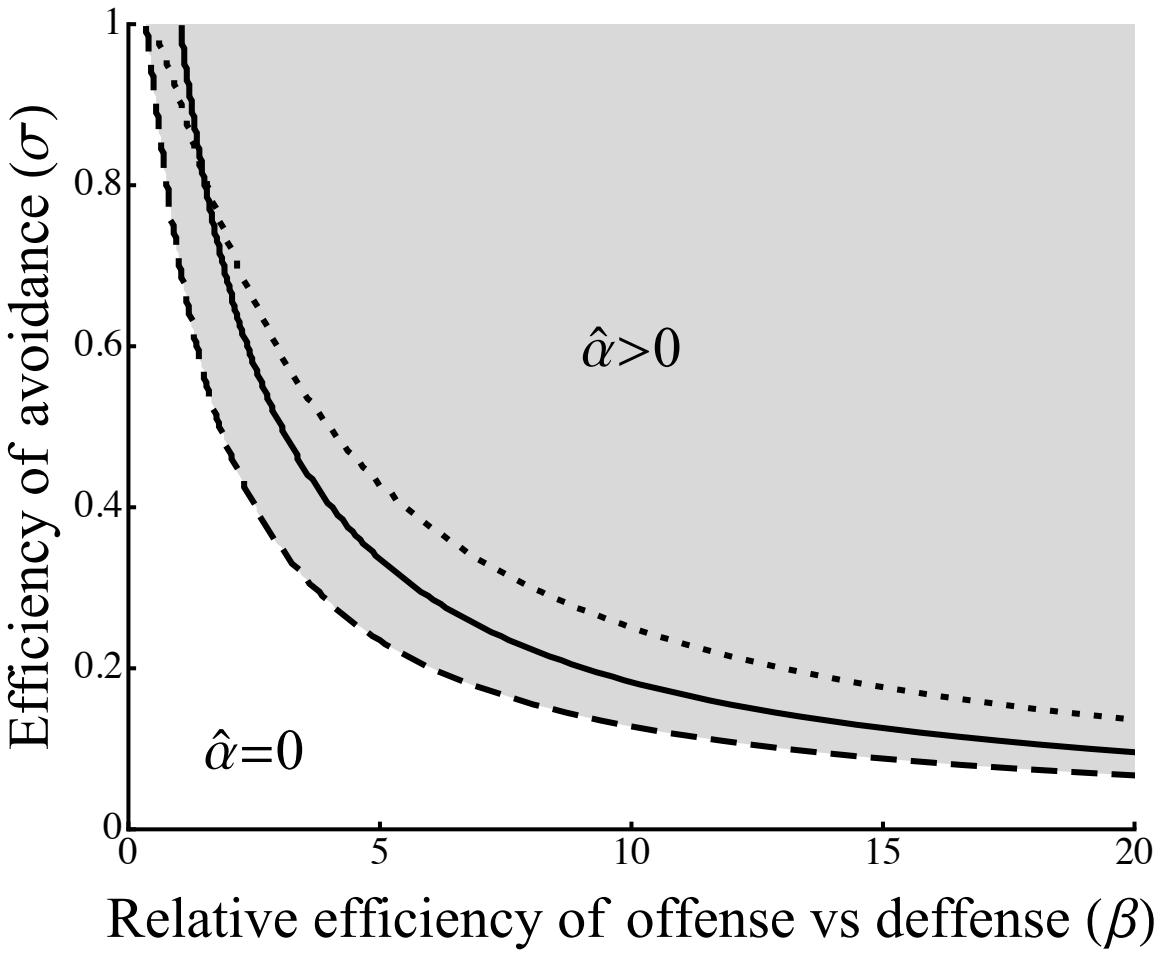

Efficiency of avoidance (σ)
1
0.8
0.6
0.4
0.2
0
$\hat{\alpha}$>0
$\hat{\alpha}$=0
0
5
10
15
20
Relative efficiency of offense vs deffense (β)

Figure 4:

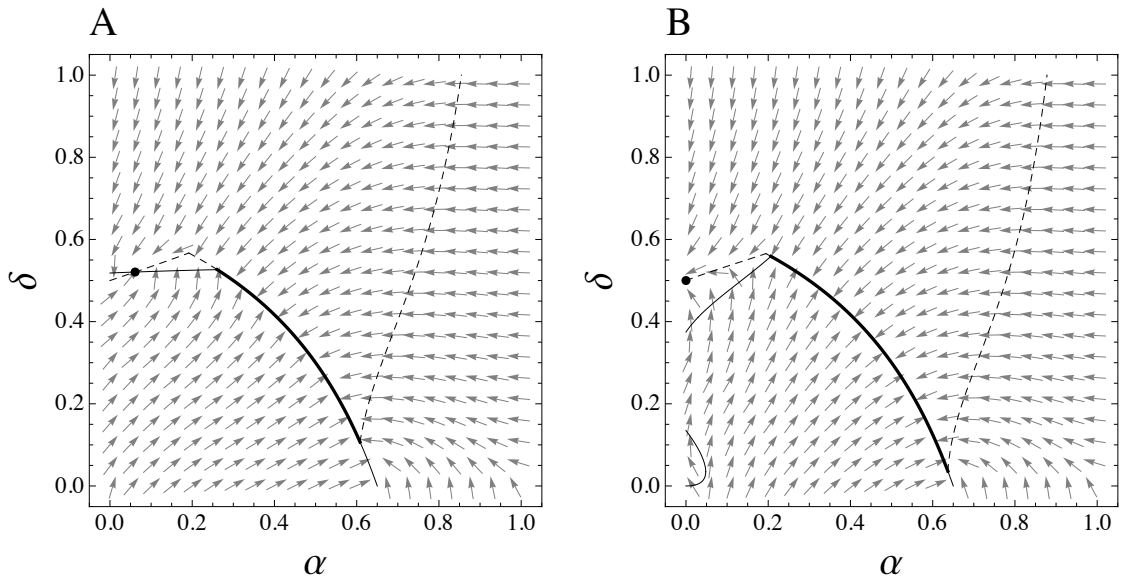


Figure 5:

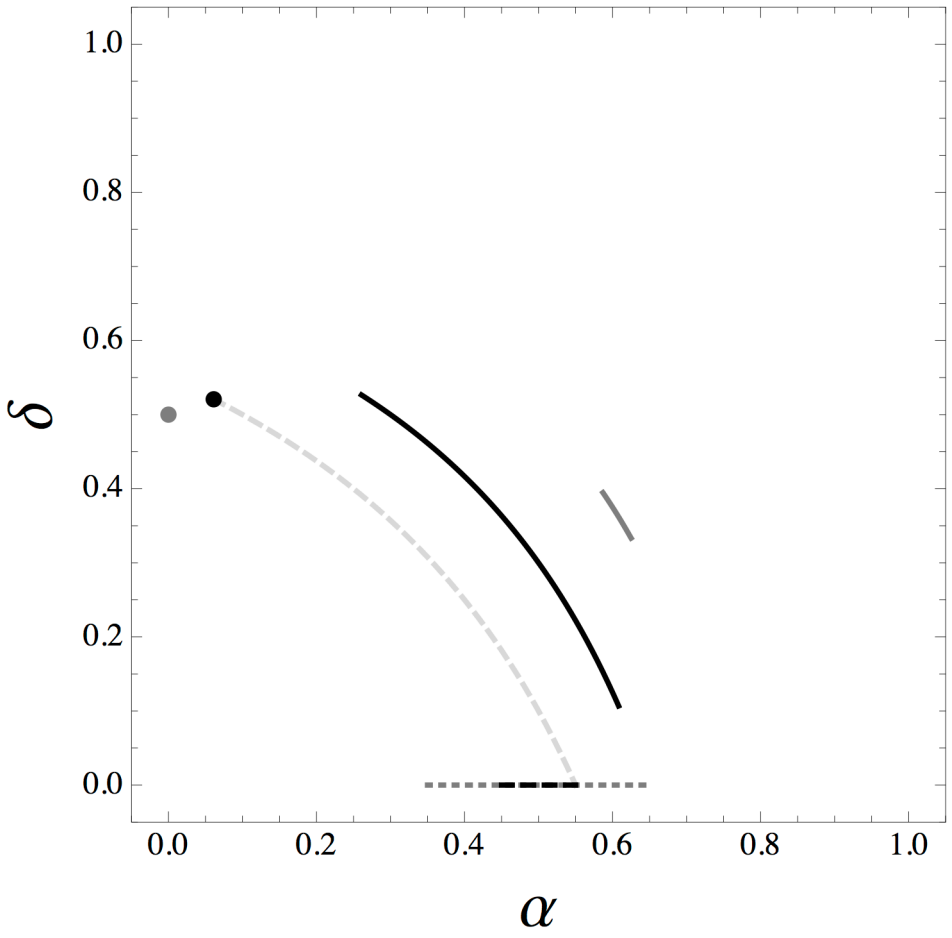
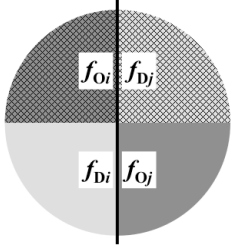
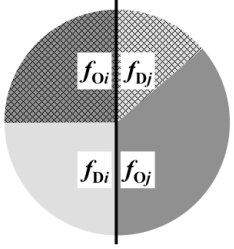
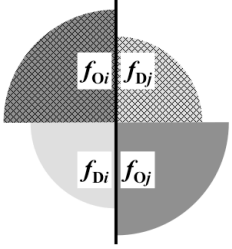
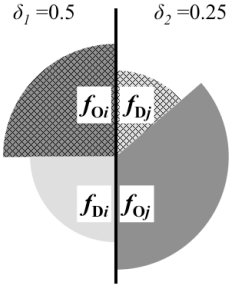


Figure 6:

	A) β **=1**		**B)** β **=2**	
	Male *i* δ_1 =0.5 Male *j* δ_1 =0.5 	Male *i* δ_1 =0.5 Male *j* δ_2 =0.25 	Male *i* δ_1 =0.5 Male *j* δ_1 =0.5 	Male *i* δ_1 =0.5 Male *j* δ_2 =0.25 
Progeny gained by male *j* through offense and defense	1st position = $\frac{f_{Dj}}{\left(f_{Dj}+\beta f_{Oi}\right)}=0.5$ 2nd position = $\frac{\beta f_{Oj}}{\left(f_{Di}+\beta f_{Oj}\right)}=0.5$	1st position = $\frac{f_{Dj}}{\left(f_{Dj}+\beta f_{Oi}\right)}=0.333$ 2nd position = $\frac{\beta f_{Oj}}{\left(f_{Di}+\beta f_{Oj}\right)}=0.6$	1st position = $\frac{f_{Dj}}{\left(f_{Dj}+\beta f_{Oi}\right)}=0.333$ 2nd position = $\frac{\beta f_{Oj}}{\left(f_{Di}+\beta f_{Oj}\right)}=0.667$	1st position = $\frac{f_{Di}}{\left(f_{Di}+\beta f_{Oi}\right)}=0.2$ 2nd position = $\frac{\beta f_{Oj}}{\left(f_{Di}+\beta f_{Oj}\right)}=0.75$
	Total progeny of male *j* = 1	Total progeny of male *j* = 0.933	Total progeny of male *j* = 1	Total progeny of male *j* = 0.95

CHAPTER V: CONCLUSIONS

The chapters in this thesis provide some new insights into the evolution of reproductive traits, but they also raise new questions. Below I summarize the conclusions and the implications of the results from each chapter. Possible avenues for future research are discussed.

In chapter 2 we address the evolution of female choosiness and of male displays that function as indicators of male quality. We show that female ability to directly detect male quality in the absence of a display can facilitate, instead of inhibiting, the evolution of indicator displays. Different displays may function as indicators of different qualities. Our results predict a pattern for what kind of male qualities may be more likely to be associated with indicator displays in nature. We predict that qualities that are detectable directly, such as resistance to diseases with detectable symptoms, body size etc., are more likely to be associated with male displays, compared to qualities that are difficult or impossible to detect independently. This prediction is important, because it may provide an explanation for why empirical evidence for indicators of good genes is not as common for certain kinds of qualities as for others.

Chapter 2 also includes a novel modeling framework for studying different indicator mechanisms. This structure highlights the continuous and non-mutually exclusive nature of the different indicator mechanisms that have traditionally been studied as discrete types.

Besides giving a more biologically realistic picture, the continuum framework allows us to compare different mechanisms more directly.

Chapter 3 concerns the empirical work done with *Drosophila pseudoobscura* on the influence of male and female age on male mating investment (Dhole and Pfennig 2014b). We find that males of intermediate ages invest more in individual matings than the youngest or the oldest males. The youngest males were found to make the smallest investments in matings. Furthermore, we found that in this species, males invest fewer resources in matings with old females compared to matings with young females. Interestingly, such discrimination is exhibited only intermediate-aged and old males, while the very youngest males make minimal investments with all females. We also find that male investment in individual matings is correlated with one aspect of fitness: early post-mating fecundity. We were unable to study other aspects of male fitness in this study, but it is possible that male mating investment influences other aspects of his reproductive performance. For example, male investment in the ejaculate often influences sperm-competitive success. Age-dependent variation in investments in ejaculates by males of different ages can make males of certain ages better sperm competitors than others. Such variation would play an important role in the evolution of traits such as female mate choice based on male age. If, for example, older males sire more progeny than young males due to sperm competition, females seeking genetic benefits through mate choice are less likely to gain those benefits through young males than old males, if females mate multiple times.

In chapter 4, the evolution of male seminal fluid composition was studied. We asked how males should invest in seminal fluid proteins that perform one of three functions: avoidance, defense or offense (Dhole and Servedio 2014). We find that the relative efficiency with which different proteins function can be an important determinant of the evolutionarily stable composition that evolves in the population. When offense proteins are more efficient than defense proteins, males mating second would have an advantage over males mating first with a female. We find that in such scenarios males evolve to invest more in avoidance Sfps. Interestingly, the relative efficiency of offense and defense influences of the evolution of investment in defense and offense only when plasticity in seminal fluid composition exists, that is, when males can facultatively adjust their seminal fluid composition based on the mating status of the female. We also find that with plasticity, multiple evolutionarily stable seminal fluid compositions can evolve across different populations. Moreover, we find that the relative viability costs of proteins also play a role in determining the seminal fluid composition that evolves in a population; male evolve to make smaller investments in more costly proteins.

Our model, to our knowledge, is the first formal treatment of how male investment in different Sfps evolves. We focused on thoroughly addressing the trade-off among different Sfps. Males in nature must allocate resources among more than just seminal fluid proteins. One interesting avenue for future research is the trade-off between different kinds of Sfps and sperm investment. Unlike the Sfps addressed here, larger investment in sperm increases sperm-competitive success in all mating contexts (Parker 1990; Birkhead and Møller 1998; Simmons 2001; Wedell et al. 2002; Parker and Pizzari 2010). Furthermore, the functions of certain Sfps are dependent on the amount of sperm that is transferred by a male. Moreover,

the trade-off between Sfps and sperm is likely to be more complex in many biological systems, because sperm and Sfps are often stored in separate organs. It is therefore likely that males have a large degree of plasticity in the relative amounts of sperm and Sfps that they transfer, compared to the plasticity in the relative amounts of different Sfps that they transfer in a given mating.

It would also be interesting to study how pre-mating sexual selection influences the optimal seminal fluid composition for different males. For example, if certain males, by virtue of their pre-mating traits, are more likely to mate with virgin females, they may benefit by increasing investments in avoidance and defense compared to investing in offense. Different optima for seminal fluid composition thus may exist for males that differ in mating success with females of different mating statuses. Preliminary modeling results (Dhole, unpublished model) suggest that merely a difference in the likelihood of mating in the first versus second position may not be sufficient to build strong genetic associations between pre-mating male traits (such as mating displays) and seminal fluid composition. Lower recombination rates between loci controlling pre- and post-mating traits, however, can facilitate stronger genetic correlations.

Finally, one potentially important aspect of sperm competition that we were not able to accommodate in our model was an evolutionary response by females. Females may evolve in response to changes in male seminal fluid composition, as certain seminal fluid proteins can reduce female viability (Ravi Ram and Wolfner 2007; Barnes et al. 2008; Fricke et al. 2009; South and Lewis 2011). Although we discuss some implications of our model for sexual conflict in chapter 4, a thorough treatment of this topic requires separate models.

The evolution of reproductive traits is complex. But that is part of the reason why it continues to fascinate generations of biologists. The broader goal of this thesis is to contribute to our shared understanding of the evolution of reproductive traits. As the field of sexual selection research continues to grow and mature, new questions keep becoming accessible to inquiry. A major avenue for future sexual selection research to understand how reproductive traits functioning at different stages of reproduction may coevolve. The chapters of this thesis contribute towards an integration of theory that addresses pre-mating sexual selection with the theory that addresses post-mating sexual selection.

APPENDIX: CHAPTER II

Appendix 2.1

Viability selection:

Males with the C_2 allele gain a viability advantage given by the parameter s_c. Males in high condition produce a full sized display if they have a T_2 allele and pay a cost given by the parameter s_t. T_2 males in poor condition may produce a display that may be smaller, of poorer quality, and may incur higher marginal costs depending upon the type of indicator mechanism. The parameter σ modifies the basal cost of the display (s_t) for the poor condition males, as shown in Table 1 in the main text.

The genotypic frequency of a genotype X_i in males after viability selection is given by

$$x'_{i_m} = \frac{\left(1+\theta s_c\right)\left(1-\zeta\sigma^{(1-\theta)}s_t\right)x_{i_m}}{\sum_i \left(1+\theta s_c\right)\left(1-\zeta\sigma^{(1-\theta)}s_t\right)x_{i_m}} \qquad \text{(A1)}$$

where x_{i_m} is the frequency of a genotype X_i in males before selection. $\theta = 1$ if i is even, and $\theta = 0$ otherwise. $\zeta = 1$ if i mod 4 is 0 or 3, and $\zeta = 0$ otherwise.

Choosy females suffer viability cost due to the cost of mate choice. The frequency of a female genotype j after viability selection is given by

$$x'_{j_f} = \frac{\left(1-\mathrm{g}s^*_{\mathrm{h}}\right)x_{j_f}}{\sum_j \left(1-\mathrm{g}s^*_{\mathrm{h}}\right)x_{j_f}} \qquad \text{(A2)}$$

where g = 0 if $j < 5$, and g = 1 otherwise. Here x_{j_f} is the frequency of genotype j in females before viability selection, and s_h^* is the weighted cost of mate choice as defined in the main text.

Sexual selection:

The frequency of matings between male genotype i and female genotype j is given by

$$M_{ij} = \frac{\left(1+\gamma^{k}\beta^{d}\omega a\right)x'_{i_m}x'_{j_f}}{\sum_i \left(1+\gamma^{k}\beta^{d}\omega a\right)x'_{i_m}} \qquad \text{(A3)}$$

where k = 1 if i is 2 or 6, and k = 0 otherwise, d = 1 if i is 3 or 7 and d = 0 otherwise, $\omega = 0$ if i is 1 or 5 or if $j < 5$, and $\omega = 1$ otherwise. The denominator in equation A3 ensures that all females that survive to reproduce have the same mating success.

Maintaining genetic variation at the C locus:

To maintain genetic variation at the C locus, we artificially hold the frequency of the C_2 allele at 0.5. We do this simply by setting the frequency of the C_2 allele to 0.5 at the end of every generation in the numerical simulations. Allelic frequencies at the other two loci and all the linkage disequilibria are allowed to evolve. This method has been previously used by Bank and colleagues (Bank et al 2012) to maintain genetic variation at one locus. The method is similar, in principle, to the mutation bias method commonly used in quantitative genetic models, in that genetic variation is added to the population without altering the correlations (linkage disequilibria) between loci or traits. One potential drawback of maintaining genetic variation using this method is that when the allelic frequency is held constant at a very high or very low value, the linkage disequilibria may become inflated or

deflated. In our model we hold the allelic frequency constant at an intermediate value of 0.5. We also compared the measures of linkage disequilibria that evolve with and without the C locus being held constant. We ran extensive simulations to compare such potential error in measures of the linkage disequilibria in our model. The amount of error varies for different linkage disequilibria, but in all cases examined, the error is less than 5%, and in a direction (inflation/deflation) that makes our biological conclusions more conservative. For example, we found that holding the C locus constant deflates linkage disequilibrium between H and C loci when condition is directly detectable, in turn, reducing the rate of spread of choosiness. Faster spread of choosiness would only strengthen our results. Using this method thus makes the simulations an approximation of exact iterations of the recursions, even if somehow variation at the C locus were to be maintained.

Appendix 2.2: Decomposition of allele frequency change

Following the assumptions of the general model of good genes processes presented in the main text, and equivalent to the equations presented in Appendix 2.1, an equation describing the fitness of each genotype can be written using multilocus notation (Barton and Turelli 1991) as

$$W(X_H X_T^* X_C X_C^*) = g_f\left((1-X_H)g_{m1} + (1-s_h^*)X_H g_{m2}\right) \qquad \text{(A4)}$$

where

$$g_{m1} = \frac{1}{\bar{w}_m}\left((1-X_T^*)(1-X_C^*) + (1+s_c)(1-X_T^*)X_C^* + (1-\sigma s_t)X_T^*(1-X_C^*) + (1-s_t)(1+s_c)X_T^* X_C^*\right),$$

$$g_{m2} = \frac{1}{\bar{w}_m z}\begin{pmatrix}(1-X_T^*)(1-X_C^*) + (1+\gamma a)(1+s_c)(1-X_T^*)X_C^* + (1+\beta a)(1-\sigma s_t)X_T^*(1-X_C^*) \\ +(1+a)(1-s_t)(1+s_c)X_T^* X_C^*\end{pmatrix},$$

$$g_f = \left((1 - X_C) + (1 + s_c)X_C\right) / \bar{w}_f ,$$

$$s_h^* = s_h - \frac{s_h z}{(1+a)} ,$$

$$\bar{w}_m = x_{T_1C_1} + (1+s_c)x_{T_1C_2} + (1-\sigma s_t)x_{T_2C_1} + (1-s_t)(1+s_c)x_{T_2C_2} ,$$

$$\bar{w}_f = 1 - s_h^* h_2 + s_c c_2 - s_h^* s_c x_{H_2C_2} , \text{ and}$$

$$z = \frac{1}{\bar{w}_m}\left(x_{T_1C_1} + (1+\gamma a)(1+s_c)x_{T_1C_2} + (1+\beta a)(1-\sigma s_t)x_{T_2C_1} + (1+a)(1-s_t)(1+s_c)x_{T_2C_2}\right) .$$

Here X_i represents the allele present in females at locus *i*, where $X_i = 0$ if allele i_1 is present and $X_i = 1$ if allele i_2 is present. Likewise X_j^* represents the allele present at locus *j* in males. Specific genotype frequencies are denoted by an *x* with the appropriate subscript, and allele frequencies are denoted in lower case. The terms g_{m1} and g_{m2} describe the fitnesses of males due to a combination of viability selection and mating by H_1 (for g_{m1}) and H_2 (for g_{m2}) females. The term g_f represents fitness determined by the condition locus in females. Weighted costs to female choosiness are represented by the selection coefficient s_h^*, as described in the main text. Note that the '*z*' in equation A4 is identical to the '*z*' described in equation 1 of the main text, but is defined here using genotype frequencies in zygotes as the point of reference. Finally, $\bar{w}_m$ and $\bar{w}_f$ are the mean fitnesses due to viability selection alone in males and females, respectively, while *z* is a normalization factor to ensure that every female has equal mating success (note that this is the sum of male genotypes weighted by how much they are preferred by females, and hence is a component of costs to choosiness). The equations, mean fitnesses, and normalization above are generally written in a parallel format (with terms grouped per genotype at the T and C loci) to increase clarity.

For this problem, coefficients describing selection on set U, $\tilde{a}_U$, can be calculated from equation (A4) following the procedure described Barton and Turelli (1991) and laid out more specifically in Kirkpatrick and Servedio (1999) (Appendix B). The change in the frequency of allele H_2 is written as

$$\Delta h = \tilde{a}_H D_{HH} + \tilde{a}_T D_{HT} + \tilde{a}_C D_{HC} + \tilde{a}_{HC} D_{HHC} + \tilde{a}_{TC} D_{HTC} \ , \qquad \text{(A5)}$$

where D_U represents the genetic association among the loci in set U. Equation (A5) can also be broken into two components,

$$\Delta h_{direct} = \tilde{a}_H D_{HH} + \tilde{a}_{HC} D_{HHC} \qquad \text{(A6a)}$$

and

$$\Delta h_{indirect} = \tilde{a}_T D_{HT} + \tilde{a}_C D_{HC} + \tilde{a}_{TC} D_{HTC} \qquad \text{(A6b)}$$

where Δh_{direct} represents evolution occurring via direct selection on locus H, through selection acting on sets of loci that contain H (the allele present at locus H thus determines fitness of these genotypes), and $\Delta h_{indirect}$ represents evolution at locus H occurring only via indirect selection, due to the genetic association of locus H with other loci in the genome (Barton and Servedio, in revision).

Likewise the change in the frequency of allele T_2 can be written as

$$\Delta t = \tilde{a}_T D_{TT} + \tilde{a}_H D_{HT} + \tilde{a}_C D_{TC} + \tilde{a}_{HC} D_{HTC} + \tilde{a}_{TC} D_{TTC} \qquad \text{(A7)}$$

with components

$$\Delta t_{direct} = \tilde{a}_T D_{TT} + \tilde{a}_{TC} D_{TTC} \qquad \text{(A8a)}$$

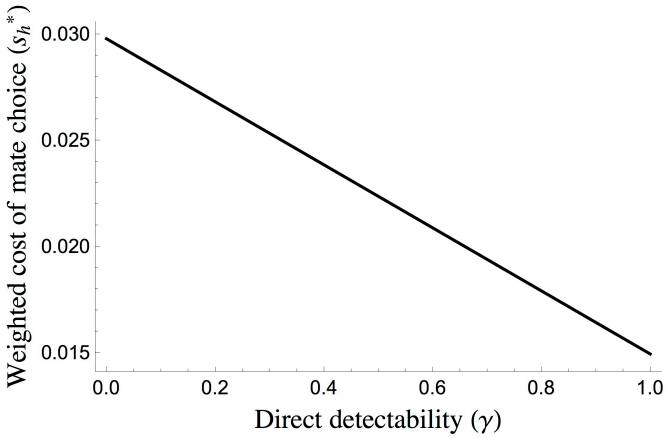
and

$$\Delta t_{indriect} = \tilde{a}_H D_{HT} + \tilde{a}_C D_{TC} + \tilde{a}_{HC} D_{HTC} \, . \qquad \text{(A8b)}$$

Calculations of the $\tilde{a}_U$ coefficients used to generate the components of Δh and Δt are available on Dryad.

Appendix 2.3: *Weighted cost of mate choice*

Weighted cost of mate choice (s_h^*) is decreases with increasing γ. Parameter values used for the graph: $a = 3$, $\beta = 0.5$, $s_h = 0.04$, display frequency = 0.01, choosiness frequency = 0.05, frequency of high condition = 0.5, all linkage disequilibria are set equal to 0.



APPENDIX: CHAPTER III

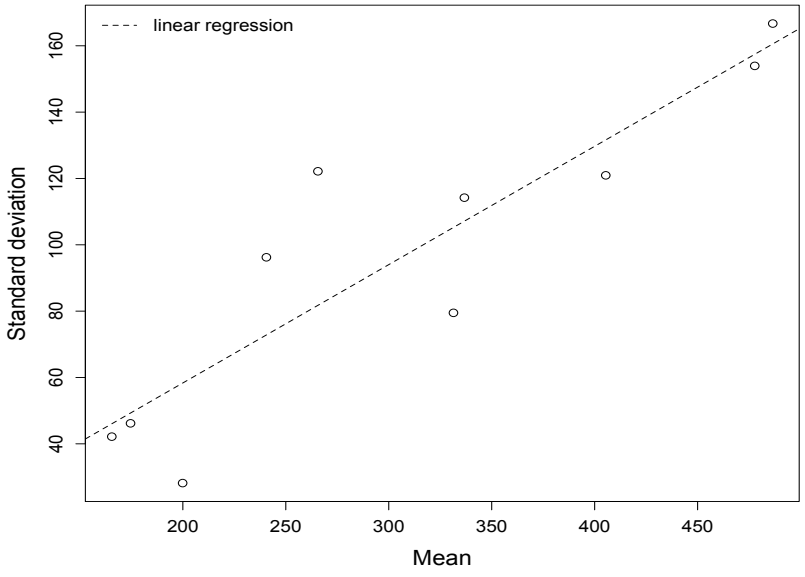
Appendix 3.1

I. Copulation Duration

Table S1: The copulation duration data are overdispersed relative to a Poisson distribution (variance to mean ratio > 1).

Female age	Male age	Mean	Variance	Variance to mean ratio
4	4	165.5	1778.0	10.7
4	8	336.7	13044.6	38.7
4	11	477.8	23697.6	49.6
4	15	486.5	27790.3	57.1
4	19	405.4	14626.7	36.1
11	4	174.6	2132.3	12.2
11	8	199.9	793.2	4.0
11	11	265.6	14922.5	56.2
11	15	240.6	9260.3	38.5
11	19	331.4	6318.3	19.1

Figure S1: Linear relationship between the mean and the standard deviation (quadratic relationship with variance) in copulation duration measured in the different male-female age combination groups.



Output for the generalized linear model:

Call:
glm(formula = Copulation.duration.seconds ~ factor(Male.age) * factor(Female.age), family = Gamma(link = log), data = copudata)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.65722	-0.21524	-0.04602	0.17905	0.79967

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.10897	0.07828	65.266	< 2e-16 ***
factor(Male.age)8	0.71030	0.12264	5.792	1.03e-07 ***
factor(Male.age)11	1.06012	0.13558	7.819	1.02e-11 ***
factor(Male.age)15	1.07827	0.12622	8.543	3.32e-13 ***
factor(Male.age)19	0.89590	0.12622	7.098	2.95e-10 ***
factor(Female.age)11	0.05353	0.12622	0.424	0.672541
factor(Male.age)8:factor(Female.age)11	-0.57498	0.18614	-3.089	0.002680 **
factor(Male.age)11:factor(Female.age)11	-0.64062	0.19491	-3.287	0.001452 **
factor(Male.age)15:factor(Female.age)11	-0.75775	0.19935	-3.801	0.000263 ***
factor(Male.age)19:factor(Female.age)11	-0.25499	0.19935	-1.279	0.204198

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.0980428)

Null deviance: 23.975 on 98 degrees of freedom
Residual deviance: 8.892 on 89 degrees of freedom
AIC: 1169.9

Number of Fisher Scoring iterations: 4

Confidence intervals:

	Estimate	2.5 %	97.5 %
(Intercept)	5.10897	4.9593716	5.2664281
factor(Male.age)8	0.71030	0.4714257	0.9527546
factor(Male.age)11	1.06012	0.7979036	1.3301991
factor(Male.age)15	1.07827	0.8329134	1.3283389
factor(Male.age)19	0.89590	0.6505508	1.1459762
factor(Female.age)11	0.05353	-0.1918258	0.3035996
factor(Male.age)8:factor(Female.age)11	-0.57498	-0.9408627	-0.2106510
factor(Male.age)11:factor(Female.age)11	-0.64062	-1.0248667	-0.2601819
factor(Male.age)15:factor(Female.age)11	-0.75775	-1.1481843	-0.3659588
factor(Male.age)19:factor(Female.age)11	-0.75775	-0.6454264	0.1367991

Type III anova of the above glm model:

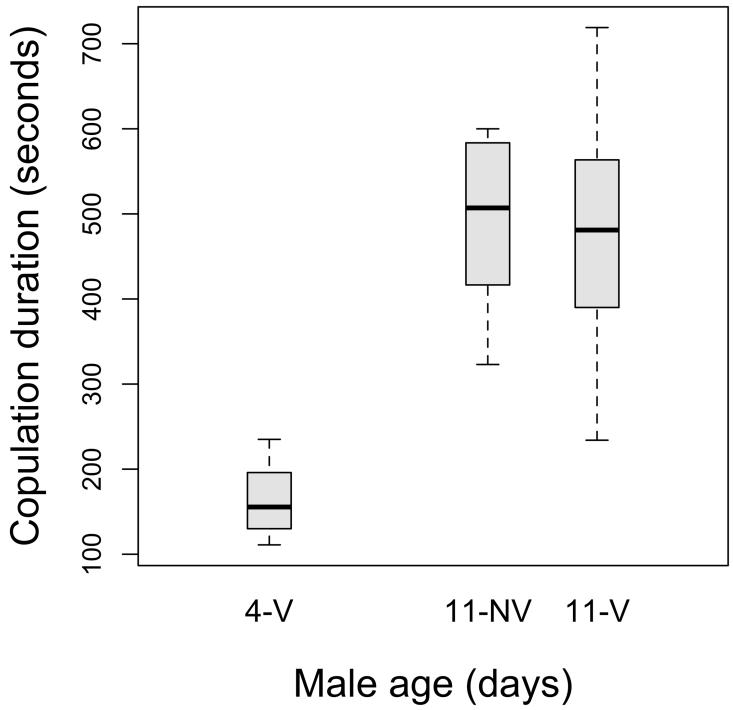
Analysis of Deviance Table (Type III tests)

Response: Copulation.duration.seconds

	LR Chisq	Df	Pr(>Chisq)
factor(Male.age)	96.010	4	< 2.2e-16 ***
factor(Female.age)	0.181	1	0.6708971
factor(Male.age):factor(Female.age)	20.663	4	0.0003693 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure S2: Copulation duration of virgin (V) and non-virgin (NV) males of two ages. Eleven-day old males copulate for longer than young 4-day old males regardless of mating status.



II. Early post-mating fecundity

Table S2: Mean-variance relationship of egg count data for all male-female mating combinations

Female age	Male age	Mean	Variance	Variance to mean ratio
4	4	78.7	1916.0	24.4
4	11	125.1	2645.5	21.1
4	19	95.0	864.0	9.1
11	4	45.6	1344.5	29.5
11	11	71.0	2082.2	29.3
11	19	53.3	1405.9	26.4

The variance to mean ratios for all the groups fall within a small range, with the exception of 4-day old females mated with 19-day old males, suggesting a linear mean-variance relationship. Models with a binomial distribution with a linear mean-variance relationship ('NB2' distribution in the GAMLSS package) were found to fit the data better than corresponding models with a negative binomial distribution with a quadratic mean-variance relationship ('NB1' distribution) or a Poisson distribution. NB1 vs NB2 vs Poisson comparisons are shown for the three best fitting models in Table S3. Results for all NB2 models are shown in Table S4.

Table S3: AICc values for the best three models with NB1, NB2 and Poisson error distributions

Distr.	Model	Predictors	K	AICc
NB2	Sans male age, sans interaction	ln(Copulation duration)+ Female age	4	576.9
NB1	Sans male age, sans interaction	ln(Copulation duration)+ Female age	4	592.4
Poisson	Sans male age, sans interaction	ln(Copulation duration)+ Female age	4	1741.5
NB2	One two-factor interactions	ln(Copulation duration)* Male age + Female age	8	577.1
NB1	One two-factor interactions	ln(Copulation duration)* Male age + Female age	8	600.8
Poisson	One two-factor interactions	ln(Copulation duration)* Male age + Female age	8	1849.8
NB2	Sans male age with interaction	ln(Copulation duration)* Female age	5	579.1
NB1	Sans male age with interaction	ln(Copulation duration)* Female age	5	594.8
Poisson	Sans male age with interaction	ln(Copulation duration)* Female age	5	1848.2

The small difference between AICc values of the two best models (Table S4) and their low Akaike weights suggest uncertainty in exclusion of the effect of male age.

However, the top four best fitting models consistently include a significant independent effect of copulation duration on early post-mating fecundity.

It should be noted that the AICc correction is designed for models with Gaussian error distribution, and the correction does not generalize in a straightforward way to models with non-Gaussian distributions. Correction for models with negative binomial distribution is not available. However, this correction is argued to be better than no correction for small sample sizes (Simonoff 2003).

Table S4: Akaike Information Criterion indices (AIC), Akaike Information Criterion indices corrected for small sample size (AICc), the number of parameters (K), Log Likelihood statististics (LL) and the Akaike weights (w) of different models are listed. The models are arranged by their AICc values.

No.	Model	Predictors	K	LL	AIC	AICc	w
1	Sans male age, sans interaction	ln(Copulation duration)+ Female age	4	–284.1	576.1	576.9	32.8%
2	One two-factor interactions	ln(Copulation duration)* Male age + Female age	8	–279.0	574.0	577.1	29.8%
3	Sans male age with interaction	ln(Copulation duration)* Female age	5	–283.9	577.8	579.1	11.1%
4	Two two-factor interactions	ln(Copulation duration)* Male age + ln(Copulation duration)* Female age	9	–278.7	575.5	579.5	9.2%
5	Main effects only	ln(Copulation duration) + Male age + Female age	6	–283.1	578.1	579.9	7.4%
6	Sans copulation duration	Male age+ Female age	5	–284.5	579.0	580.2	6.4%
7	One two-factor interactions	ln(Copulation duration)* Female age + Male age	7	–282.8	579.6	582.0	2.6%
8	Three two-factor interactions	ln(Copulation duration)* Male age + ln(Copulation duration)* Female age + Male age*Female age	11	–278.3	578.5	584.7	0.7%
9	Female age only	Female age	3	–290.2	586.4	586.8	0.2%
10	3-factor interaction	ln(Copulation duration)* Male age* Female age	13	–277.1	580.3	589.1	0.1%

A summary of the two best models is shown below.
(Note: In gamlss models, "log" refers to the natural log.)

Best model:

****

Family: c("NBII", "Negative Binomial type II")

Call: gamlss(formula = Eggs ~ log(Copulation.duration.seconds) + factor(Female.age), family = NBII, data = eggdata)

Fitting method: RS()

Mu link function: log

Mu Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.9157	1.0496	0.8725	0.3870502
log(Copulation.duration.seconds)	0.6459	0.1794	3.5993	0.0007213
factor(Female.age)11	-0.5451	0.2113	-2.5801	0.0127990

Sigma link function: log
Sigma Coefficients:

Estimate	Std. Error	t value	Pr(>|t|)
3.908e+00	2.291e-01	1.706e+01	1.018e-22

No. of observations in the fit: 55
Degrees of Freedom for the fit: 4
Residual Deg. of Freedom: 51
at cycle: 5

Global Deviance: 568.1132
AIC: 576.1132
SBC: 584.1425
****

Confidence intervals:

	Estimate	2.5 %	97.5 %
(Intercept)	0.9157	-1.1571737	2.9886597
log(Copulation.duration.seconds)	0.6459	0.2909656	1.0008074
factor(Female.age)11	-0.54511	-0.9606749	-0.1295458

Second best model:

****
Family: c("NBII", "Negative Binomial type II")
Call: gamlss(formula = Eggs ~ log(Copulation.duration.seconds) * factor(Male.age) + factor(Female.age), family = NBII)

Fitting method: RS()

Mu link function: log
Mu Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-7.589	3.6375	-2.086	0.042390
log(Copulation.duration.seconds)	2.298	0.6944	3.309	0.001804*
factor(Male.age)11	11.868	4.1556	2.856	0.006370*
factor(Male.age)19	11.660	5.9299	1.966	0.055176
factor(Female.age)11	-0.713	0.2289	-3.114	0.003137*
log(Copulation.duration.seconds):factor(Male.age)11	-2.182	0.7758	-2.813	0.007146*
log(Copulation.duration.seconds):factor(Male.age)19	-2.202	1.0505	-2.097	0.041436*

Sigma link function: log

Sigma Coefficients:

Estimate	Std. Error	t value	Pr(>|t|)
3.752e+00	2.283e-01	1.643e+01	4.128e-21

No. of observations in the fit: 55
Degrees of Freedom for the fit: 8
Residual Deg. of Freedom: 47
at cycle: 4

Global Deviance: 557.9931
AIC: 573.9931
SBC: 590.0518

****

Confidence intervals:

	Estimate	2.5 %	97.5 %
(Intercept)	-7.589	-14.70528089	-0.4735016
log(Copulation.duration.seconds)	2.298	0.93725102	3.6582401
factor(Male.age)11	11.868	3.78774969	19.9482951
factor(Male.age)19	11.660	-0.04658854	23.3673123
factor(Female.age)11	-0.713	-1.16331291	-0.2626987
log(Copulation.duration.seconds):factor(Male.age)11	-2.182	-3.68557690	-0.6786359
log(Copulation.duration.seconds):factor(Male.age)19	-2.202	-4.26885266	-0.1360741

References:

Simonoff JS (2003) Analyzing Categorical Data. New York: Springer-Verlag.

APPENDIX: CHAPTER IV

Appendix 4.1 - Non-linear avoidance effects:

To simulate the non-linear relationships between a male's avoidance investment and the fraction of his mate's progeny that is produced before remating (see Figure 1 in main text), we used cumulative distribution functions (CDFs) for two distributions. Using *Mathematica* (Wolfram 2011), a sigmoid relationship was simulated using the CDF for a truncated Normal distribution with mean μ and standard deviation τ, and a truncated Half-normal distribution with the scaling parameter θ was used to simulate a concave relationship. *Mathematica* code for these analyses is provided in the online supplementary files. The fraction of progeny produced before remating when a female mates first with a male of genotype g is given by $C_{\mathrm{A}g}\sigma$, where $C_{\mathrm{A}g}$ is the value of the CDF evaluated at $f_{\mathrm{A}g}$. Male progeny payoffs are calculated similar to the case of linear avoidance effects after modifying equation 2 as

$$\mathrm{m}_{gh} = C_{\mathrm{A}g}\sigma + \left(1 - C_{\mathrm{A}g}\sigma\right)\frac{f_{\mathrm{D}g}}{f_{\mathrm{D}g} - f_{\mathrm{O}h}\beta}. \qquad \text{……(A1)}$$

Appendix 4.2 - Analysis of the basic model with linear avoidance effects

The recursion equations for the allele frequencies are used to perform linear stability analyses. Two of the eigenvectors of the Jacobian matrix of the system of equations are collinear with the axes of allelic frequencies at the two loci. Each of the eigenvalues thus represents the invasion fitness of a new rare allele relative to the resident allele at the corresponding locus. The direction of evolution at the two loci is thus controlled independently by the corresponding eigenvalues.

Evolution at the D locus

When a genotype A_iD_l is fixed in the population the eigenvalue corresponding to stability along the axis described by the allelic frequency at the D locus simplifies to

$$\frac{1}{2}\left(2-\mathrm{m}_{gh}+\mathrm{m}_{hg}\right), \qquad \ldots\ldots(A2)$$

where the genotype A_iD_l is denoted by '*g*', the genotype of the competitor A_iD_k is denoted by '*h*' and m_{gh} is the paternity share of a male with genotype *g* when mating in the first position when a male with genotype *h* is mating second (see equation 2 in the main text). This simplifies to give condition (7) in the main text for the fixation and stability of the equilibria at the D locus.

Evolution at the A locus

Similarly to evolution at the D locus, when the genotype A_iD_l is fixed in the population, the eigenvalue corresponding to stability along the axis described by the allelic frequency at the A locus simplifies to

$$\frac{1}{2}\left(2-\mathrm{m}_{gf}+\mathrm{m}_{fg}\right), \qquad \ldots\ldots(A3)$$

where the genotype A_iD_l is denoted by '*g*', the genotype of the competitor A_jD_l is denoted by '*f*' and m_{gf} is the paternity share of a male with genotype *g* when mating in the first position while a male with genotype *f* is mating second (see equation 2 in main text).

As described in the main text, in the basic model the D locus will eventually be fixed for an allele with a phenotypic value of $\hat{\delta} = 0.5$ irrespective of the dynamics at the A locus. Therefore, we analyze stability at the A locus with phenotypic value of the D_l allele fixed at $\delta = 0.5$.

We find that an A_j allele (with phenotypic value α_j) will increase in frequency over an allele A_i (with phenotypic value α_i) and remain fixed in the population if one of the following conditions is satisfied-

$$\frac{2-\left(1+\beta\right)\sigma+\alpha_i\left(\beta\sigma-1\right)}{1+\alpha_i\left(\beta-1\right)\sigma-\beta\sigma}<\alpha_j<\alpha_i$$

or ……(A4)

$$\alpha_i<\alpha_j<\frac{2-\left(1+\beta\right)\sigma+\alpha_i\left(\beta\sigma-1\right)}{1+\alpha_i\left(\beta-1\right)\sigma-\beta\sigma}.$$

The quantity on the extreme left hand side in the first condition (extreme right in the second condition) is an involutary function, $f(\alpha_i)$, of α_i, i.e. $f(f(\alpha_i)) = \alpha_i$. It describes the phenotypic value of an allele with fitness equal to that of the A_i allele (see Figure S1 for an example). An allele that has a phenotypic value α_i, such that $f(\alpha_i)= \alpha_i$, will be the convergent stable value in the system. This phenotypic value gives the amount of investment that will eventually evolve to be fixed in the population, and is given by $\hat{\alpha}$ as described by equation 8 in the main text.

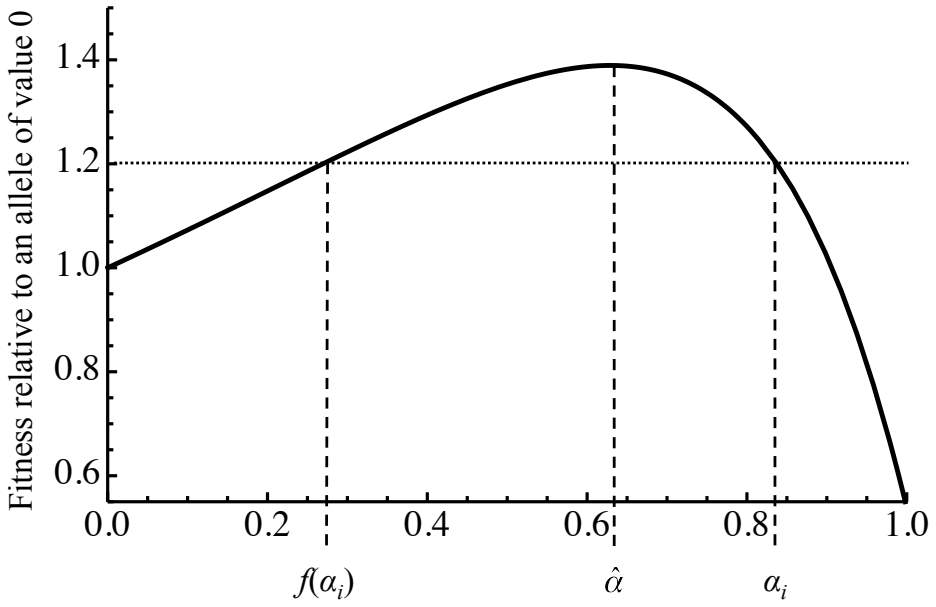


Figure S1: The fitnesses (on the *y* axis) of all possible alleles at the A locus (phenotypic values on the *x* axis) relative to an allele that codes for zero investment in avoidance. $\beta = 6$, $\sigma = 0.7$.

Testing for internal equilibria

We used numerical simulations to check for the presence of polymorphic equilibria. We assume that the optimal alleles for each of the two loci are present in the population or will arise eventually though mutation. Therefore, we tested whether a polymorphic equilibrium can be achieved with one of the two competing alleles at each of the loci set at the optimal phenotypic value ($\hat{\alpha}$ and $\hat{\delta}$), while the other allele (α_i and δ_k) could take any of the values given below. All combinations of the following values for the parameters and for the competing alleles were used for simulations.

δ_k: 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 0.99

α_i: 0.1, 0.2, 0.3, 0.35, 0.4, 0.5, 0.55, 0.6, 0.7, 0.8, 0.9, 0.99

β: 0.1, 0.2, 0.25, 0.3, 0.4, 0.5, 0.7, 0.9, 1, 2, 3, 4, 10

σ: 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8, 0.9, 0.999

These values give a total of 10,920 combinations. Simulations were run until an equilibrium was reached. This was assumed to occur when the change in allelic frequencies between successive generations was less than $1x10^{-11}$. No indications of polymorphic equilibria were observed in any of the simulations.

Appendix 4.3 - Alternative forms of defense and offense Sfp interaction

The interaction between defense and offense Sfps given in equation 2 in the main text describes a linear increase in the effect of the proteins with increasing investment in them, i.e. each additional protein molecule is expected to contribute equally to the interaction. Currently we do not have sufficient empirical information on how these proteins interact in actual organisms, but we do not have any a priori reasons to suspect that their effects increase non-linearly. However, given the possibility that the effect of each additional unit of investment in offense and defense proteins may depend upon the total investment in these proteins, we analyzed two forms of non-linear effects. We consider cases when each additional unit of investment in these proteins has a progressively higher or progressively lower effect. These effects were modeled by the following modifications of equation (2) in the main text.

Faster than linear increase

$$m_{gh} = f_{\mathrm{Ag}}\sigma + \left(1 - f_{\mathrm{Ag}}\sigma\right)\frac{f_{\mathrm{Dg}}^2}{f_{\mathrm{Dg}}^2 + f_{\mathrm{O}h}^2\beta} \qquad \ldots\ldots(\mathrm{A5})$$

Slower than linear increase

$$m_{gh} = f_{\mathrm{Ag}}\sigma + \left(1 - f_{\mathrm{Ag}}\sigma\right)\frac{\sqrt{f_{\mathrm{Dg}}}}{\sqrt{f_{\mathrm{Dg}}} + \sqrt{f_{\mathrm{Oh}}}\beta} \qquad \text{……(A6)}$$

We performed linear stability analysis to study evolution at the D locus with these forms of interactions similar to the analysis described above in Appendix 4.2. We find that even with these non-linear interactions, equal investment in defense and offense proteins results in higher fitness than any unequal investment. Also, the optimal investment in avoidance proteins remains unaffected by these modifications of the interaction between defense and offense proteins. We do note that if the offense and defense proteins obey different forms of increase from one another in their effects, this result may not hold true. However, without further information on the biochemistry of these protein interactions, there is no reason to believe this is the case.

Appendix 4.4 - Alternative resource reallocation structure

In the models described in the main text, the A locus separately determines resources out of the total that are allocated to avoidance, and the remaining resources are divided into defense and offense by the second locus D. We analyze the two alternative ways for allocating resources into three kinds of Sfps with two loci – separate offense allocation, and separate defense allocation. We describe details of the analysis for separate offense allocation here. Separate defense allocation follows a similar analysis, details of which can be found in the supplementary *Mathematica* file.

For separate offense allocation, the D locus determines the fraction of total resources that are allocated to offense proteins. The remaining resources are divided into avoidance and defense proteins. In parallel to Expression (1) of the main text, the fractions of the *total* resources of a male of genotype D_iA_k that go towards each category of Sfps produced with this alternative structure are thus

$$\begin{aligned} &\text{Offense investment } (f_O)\text{: } \delta_i \\ &\text{Avoidance investment } (f_A)\text{: } (1-\delta_i)\,\alpha_k \\ &\text{Defense investment } (f_D)\text{: } (1-\delta_i)\,(1-\alpha_k) \end{aligned} \qquad \ldots\ldots(A7)$$

Note that with this notation, higher allelic values at the D locus results in higher offense investment, and higher allelic values at the A locus result in more of the remaining resources going towards avoidance and fewer towards defense. As described in Appendix 4.2, the eigenvalues of the system of recursion equations represent the invasion fitness of a rare new allele relative to the allele that is fixed in the population. These expressions are used to determine the optimal allelic value at each locus (α or δ) when the other locus is fixed for a certain allelic value. The joint optima for the two loci give the optimal seminal fluid composition. We use sequential invasion analysis to determine whether the optimal seminal fluid composition is convergent-stable.

When the A locus is fixed for an allele A_k (with allelic value α_k), and the D locus is fixed for an allele D_i (with allelic value δ_i), a new allele D_j (with allelic value δ_j) can increase in frequency and spread in the population if one of the following conditions is satisfied-

$$\frac{\left(\alpha_k-1\right)\left(\delta_i-1\right)\left(\alpha_k\sigma-1\right)}{\alpha_k^2\left(\delta_i-1\right)\sigma+\alpha_k\left(1-\sigma\left(\beta\delta_i+\delta_i-1\right)\right)-1}<\delta_j<\delta_i$$

or ……(A8)

$$\delta_i<\delta_j<\frac{\left(\alpha_k-1\right)\left(\delta_i-1\right)\left(\alpha_k\sigma-1\right)}{\alpha_k^2\left(\delta_i-1\right)\sigma+\alpha_k\left(1-\sigma\left(\beta\delta_i+\delta_i-1\right)\right)-1}.$$

The quantity on the extreme left in the first (extreme right in the second) inequality in A8 is an involuntary function of δ_i. The conditions simplify to give the ESS value for the D locus allele when an allele A_k is fixed in the population,

$$\delta=\frac{\alpha_k^2\sigma-\sqrt{\left(\alpha_k-1\right)\left(\alpha_k\sigma-1\right)\left(\alpha_k\left(\beta\sigma-1\right)+1\right)}-\alpha_k\left(\sigma+1\right)+1}{\alpha_k\sigma\left(\alpha_k-\beta-1\right)} \qquad \text{……(A9)}$$

When the D locus is fixed for an allele D_i, and the A locus is fixed for an allele A_k, a new allele A_l can increase in frequency and spread in the population if

$$\alpha_l<\alpha_k \text{ and } \sigma<\frac{1}{1-\delta_i+\beta\delta_i}$$

or ……(A10)

$$\alpha_k<\alpha_l \text{ and } \frac{1}{1-\delta_i+\beta\delta_i}<\sigma.$$

The A locus is neutrally stable when

$$\frac{1}{1-\delta_i+\beta\delta_i}=\sigma. \qquad \text{……(A11)}$$

Equations A9 and A11 together give the joint optima for allelic values α and δ at the two loci,

$$\delta_{opt} = \frac{1-\sigma}{\beta\sigma - \sigma} \qquad \ldots\ldots(A12)$$

$$\alpha_{\text{opt}} = \frac{\sigma - 2 + \beta\sigma}{\beta\sigma - 1} \qquad \ldots\ldots(A13)$$

The seminal fluid composition described by these optimal values is identical to the seminal fluid composition described by the optimal values for our original resource allocation structure (expressions 7 and 8 in the main text).

We use sequential invasion analysis to determine if this optimal combination of allelic values is convergent-stable. We do this by evaluating the eigenvalues of the matrix

$$\left(\begin{array}{cc} \frac{\partial}{\partial\delta}\left(\frac{\partial\lambda_1}{\partial\delta_{\text{m}}}\Big|_{\delta_{\text{m}}=\delta} \right) & \frac{\partial}{\partial\alpha}\left(\frac{\partial\lambda_1}{\partial\delta_{\text{m}}}\Big|_{\delta_{\text{m}}=\delta} \right) \\ \frac{\partial}{\partial\delta}\left(\frac{\partial\lambda_2}{\partial\alpha_{\text{m}}}\Big|_{\alpha_{\text{m}}=\alpha} \right) & \frac{\partial}{\partial\alpha}\left(\frac{\partial\lambda_2}{\partial\alpha_{\text{m}}}\Big|_{\alpha_{\text{m}}=\alpha} \right) \end{array} \right)_{\substack{\delta=\delta_{\text{opt}} \\ \alpha=\alpha_{\text{opt}}}} \qquad \ldots\ldots(A14)$$

where λ_1 is the invasion fitness of a rare mutant allele at the D locus with value δ_{m} relative to the resident allele at the D locus when the A locus is fixed with an allele. Likewise λ_2 is the invasion fitness of a rare mutant at the A locus with value α_{m} relative to the resident allele at the A locus when the D locus is fixed with an allele. As described in Appendix 4.2, these invasion fitnesses are the eigenvalues of the Jacobian of the system of recursions of allelic frequencies and the disequilibrium. All eigenvalues of the above matrix evaluated at the optimal combination allelic values (eqn A12 and A13) have negative real parts, indicating local convergence (see supplementary *Mathematica* file).

Appendix 4.5 - Plasticity in Sfp investment

We use sequential invasion analysis to determine the combinations of allelic values at the two loci A and D that, when fixed in the population, cannot be invaded by new rare alleles. Unlike the model without plasticity where we find a single convergent stable allelic combination for a given set of parametric values, with plasticity we find that multiple combinations of allelic values at the two loci can be evolutionarily stable when invading alleles are rare. The invasion analysis gives expressions for independent evolutionary stable strategies for each of the loci. Joint ESSs are given by allelic values that satisfy the conditions for evolutionary stability at both loci. These results are also robust to changing the resource allocation structure (evolutionarily stable seminal fluid compositions were tested with the separate offense allocation structure in addition to the separate avoidance structure described above).

The direction of the arrows shown in figures 4 and 5 in the main text is given by the vector

$$\left\{\left(\left.\frac{\partial\lambda_2}{\partial\alpha_{\mathrm{m}}}\right|_{\alpha_{\mathrm{m}}=\alpha}\right),\left(\left.\frac{\partial\lambda_1}{\partial\delta_{\mathrm{m}}}\right|_{\delta_{\mathrm{m}}=\delta}\right)\right\} \qquad \ldots\ldots(\mathrm{A15})$$

evaluated at values of α and δ at the base of the arrow. The lengths of the arrows was set to be equal for visual clarity. Here λ_1 is the invasion fitness of a rare mutant allele at the D locus with value δ_{m} relative to the resident allele at the D locus when the A locus is fixed with an allele with value α. Likewise λ_2 is the invasion fitness of a rare mutant at the A locus with value α_{m} relative to the resident allele at the A locus when the D locus is fixed with an allele with value δ.

The curves of critical points for the A and D loci are given by the following equations respectively-

$$\left(\frac{\partial \lambda_2}{\partial \alpha_m}\bigg|_{\alpha_m=\alpha} \right) = 0 \qquad \text{……(A16)}$$

$$\left(\frac{\partial \lambda_1}{\partial \delta_m}\bigg|_{\delta_m=\delta} \right) = 0 . \qquad \text{……(A17)}$$

Appendix 4.6 – Viability selection

First we determine the optimal allelic value for the D locus for a given combination of viability costs of the three kinds of proteins with the A locus is fixed for an allele. As the viability costs of defense and offense proteins become unequal, males evolve to invest fewer resources in the costlier Sfp (Figure S2).

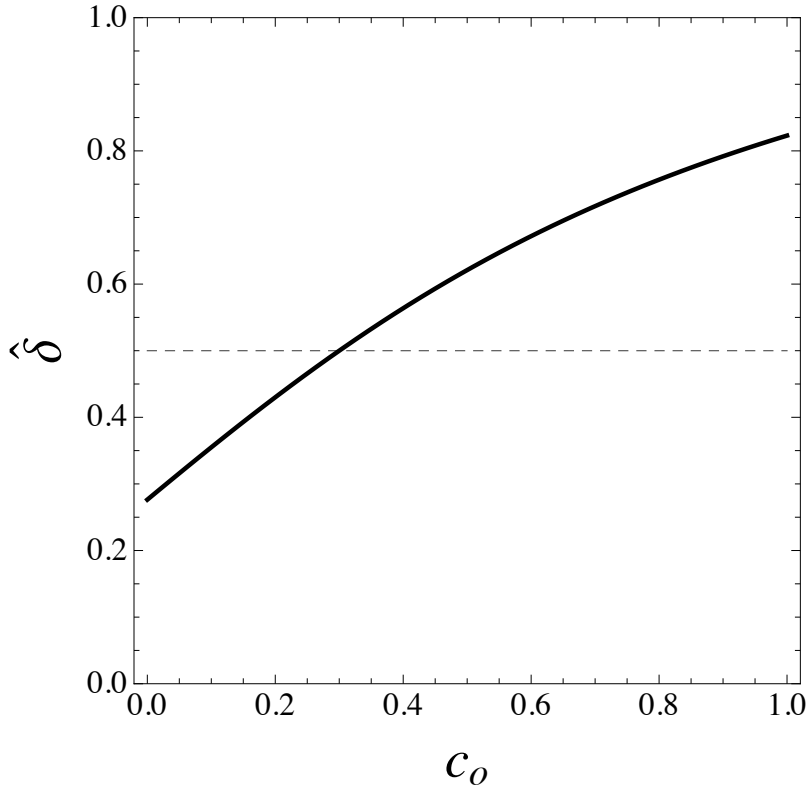


Figure S2: Optimal allelic value for the D locus (solid black line) is plotted against the viability cost of offense proteins (c_o). The cost of defense (c_d) and avoidance (c_a) proteins are set at 0.3. The allelic value that gives equal investment in defense and offense (δ=0.5) is shown for reference (dashed gray line). The A locus is fixed for an allele with value α =0.4. β=2; σ=0.7.

Similar to the D locus, we calculate the optimal investment in avoidance for a given combination of viability costs of the three Sfps. A higher viability cost of avoidance results in evolution of lower investment in avoidance (Figure S3). Also, we find that higher investment in avoidance can be maintained for a given cost as the advantage to the second male (β) increases (Figure S3).

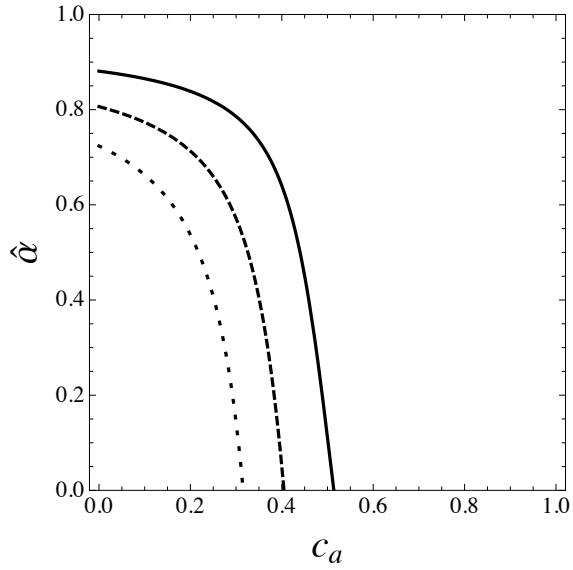


Figure S3: Optimal allelic values for the A locus are plotted against the viability cost of avoidance proteins (c_a). The cost of defense (c_d) and offense (c_o) proteins are set at 0.3. The D locus is fixed for an allele with value δ =0.5. The three curves show optimal allelic values at different levels of advantage to the second male: dotted line - β=2; dashed line - β=3; solid line - β=5. Efficiency of avoidance proteins, σ=0.7.

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